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A RECORD OF RESEARCH CARRIED ON
IN VARIOUS BRANCHES OF SCIENCE

Vol. VII, 1931-32

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LIFE MOVEMENTS

I.—INTRODUCTORY

BY

SIR J. C. BOSE, F.R.S.

THE present volume marks a new departure, in that, unlike its predecessors, it includes papers relating to Zoology and Anthropology. The scope of investigation being thus greatly enlarged, the title 'Life Movements of Plants' hitherto attached to the publication now becomes inappropriate and is accordingly dropped.

Among the important results secured in the Institute during the last year may be mentioned the satisfactory explanation of the method of catching fish by the addition of certain plant extracts to the water in hill streams. Special automatic recorders were perfected which gave a continuous record of the physiological changes induced in the fish during the whole process. The results show that the extract induces inactivation of the respiratory mechanism, death of the fish being due to asphyxiation.

Another series of observations is described on the motor paralysis which is induced in the fish by local application of salt, the effect of which is transmitted to a distance with unequal speed in the longitudinal and in the transverse direction.

Continued investigation on the conduction of excitatory impulse in the leaf of sensitive plants like the *Mimosa* demonstrates the existence of definite conducting channels between the central and peripheral ends of the leaf. Impulses

can be made to travel along these either in a centripetal or in a centrifugal direction, according as the stimulus is applied at the central or at the peripheral end of the leaf.

New results have also been obtained on the combined effects of direct and indirect stimulation. It has been possible to obtain interesting results on the additive and differential effects of the two reactions, which can be made to act either in concordance or in opposition. These additive or differential effects are discerned from the results of characteristic mechanical responses.

The effect of homodromous and heterodromous electric current on transmission of excitation in plants has been determined by a new and comparatively simple method.

The existence of special impulse-conducting strands in the petiole of *Mimosa* has been demonstrated by the electric detection of the effect of centripetal impulse from the periphery to the centre, and of the centrifugal impulse from the centre to the periphery.

The modifying effect of change of environment on the irritability of *Neptunia oleracea* has been fully demonstrated. The environment as well as the habitat is found to play an important part in modifying the activity of the organism. The various manifestations of irritability, such as the contractility, the apex time, the latent period, and the power of conduction of excitation, are found to be appreciably modified.

An isotropic organ is shown to be rendered anisotropic by differential action of stimulus on its opposite sides. The induction of anisotropy by the differential action of light has been demonstrated as well as by the stimulus of gravity. The induced anisotropy was detected by the method of electric response.

The growth of plants undergoes modification under different modes of external stimulation. The result is found to be complicated by several factors, the individual effect of which has been determined. In regard to photic stimulus, the modifying factors are the quality or colour of light, the energy-content of the tissue, and the point of application of the stimulus constituting direct or indirect stimulation. In

regard to condition of subtonicity, the response to light is an acceleration of growth instead of the normal retardation. In extreme cases of subtonicity, as also by the effect of age, growth of the organ is brought to a state of standstill. This arrested growth is found to be revived under the stimulus of light. By the very sensitive Method of Balance the relative effects of different kinds of light have been determined with great accuracy. Though red light, in general, is ineffective in inducing any variation of growth, yet under certain circumstances it is found to induce an enhancement of the rate of growth. In regard to the modification of growth induced by direct and indirect stimulation, the effects are found to be opposite to each other. Direct stimulation induces the normal retardation, while indirect stimulation brings about an enhancement of the rate of growth. The normal action of light in retarding growth is probably due to its action as a stimulus, for a non-photic mode of stimulation, such as the electric, causes essentially similar results.

The characteristic effects of traces of indigenous plant extracts on different organs of animals have also been investigated, and the results described.

An account of fish-eating spiders of Bengal is given, and instantaneous photographs have been obtained of the method of capturing their prey, as well as that of the fertilisation of the female.

In the department of chemistry further investigations have been carried out on Proteolytic Enzymes, and on the chemical constitution of oils from Leguminous Pulses. The examination of the oils has yielded important results, indicating the presence of carotinoids.

The work of the newly-founded Department of Anthropology and Racial Biology is represented by a paper on Burmese crania. These subjects are of the greatest significance, and India offers a unique field for the systematic investigation of the biological problems relating to man. Here are to be found racial elements of diverse character, living side by side in various degrees of admixture. But, unfortunately, amongst the most primitive races at the present time a rapid depopulation is taking place, and some

of them are fast disappearing in the struggle for existence. Realising this, investigations have been commenced for the determination of the basic relationships of the different groups of Indian people. The paper published in this volume is the first of a series, in which an attempt is made to determine the norm of the Mongoloid racial type by examining a group of authentic Burmese crania. In subsequent papers the results of similar investigations, specially on the Aborigines, as well as on other groups, will be recorded. The results of these investigations, it is hoped, will supply definite materials for the pursuit of the more complicated problems affecting the biology of the people of India.

The volume concludes with an account of an investigation on the Radio-activity of the hot springs in the ancient seat of pilgrimage at Rajgir.

CALCUTTA, *May 1932.*

II.—CAPTURE OF FISH BY DRUGGING A STREAM

BY

SIR J. C. BOSE, F.R.S.

IN the hill-stream of Darjeeling large quantities of fish are captured by the Lepcha fishermen, who apply certain vegetable extracts, derived from stems, fruits, and roots, to the water. These agents are supposed to 'poison' the fishes since, after prolonged action, they cause their death. Even before this final stage is reached, the fishes, becoming moribund, are carried passively down the stream, when it is easy to collect them.

This method of capturing fish by 'poisoning' has been independently discovered and practised by many primitive peoples all over the world. But, on account of its great destructiveness, legislation has been invoked to discontinue the practice wherever possible.

A representative list of plants, the extracts from which are used for capturing the fish, is given below.¹

STEM AND BARK : *Bassia butyracea*, Roxb. ; *Crotalaria paniculata*, Willd. ; *Derris elliptica*, Benth. ; *Fluggea Leucopyrus*, Willd. ; *Lasiosiphon eriocephalus*, Dcne. ; *Millettia piscidia*, W. & A. ; *Mundulea suberosa*, Benth. ; *Berberis aristata*, D.C.

¹ It has been suggested that the death of the fish is due to the presence of saponin in the plant-extract ; and that its solvent action on blood corpuscles producing hæmolysis is in some way the cause of the death of the fish. It may be stated in this connection that there are plant-extracts which do not contain saponin, which nevertheless act as fish-poison. The present investigation has for its object the discovery of the physiological action of such extracts and other agents, which cause the death of the fish, as well as the tracing of the particular physiological mechanism, the failure of which brings about the fatal issue.

SEEDS AND FRUITS : *Anamirta Cocculus*, W. & A. ;
Barringtonia racemosa, Roxb. ; *Diospyros montana*,
Roxb. ; *Gynocardia odorata*, R.Br. ; *Hydnocarpus*
venenata, Gaertn. ; *Randia dumetorum*, Lamk. ;
Sapium indicum, Willd. ; *Spilanthes Acmella*, Linn.
ROOT : *Millettia pachycarpa*, Benth.

METHOD OF PROCEDURE FOR FISH-CAPTURE

The following account relates to the action of extract from the root of *Millettia pachycarpa*, which is a leguminous climbing plant, found in the Himalayan forest up to a height of 4000 feet and extensively used by the hill-people, the Lepchas, for capturing the fish. The root is dug up and pounded, and a solution made which is poured into the stream. A dyke with a narrow opening is usually made in the shallow portion of the stream. Various kinds of fish, becoming affected, lose their normal balance ; they wobble about and ultimately lie passively upside-down on their backs. Floating or half sunk, in this inert condition, they are carried by the flowing stream, and are easily captured at the narrow channel.

It is popularly believed that it is the severe irritation of the eye, brought about by the action of the extract, that causes the death of the fish. This supposition will, however, be shown to be without any foundation.

The fish, generally supposed to be poisoned by the root-extract, is, nevertheless, placed in the market and utilised as human food without producing the slightest evil effect. One is thus confronted with the anomaly of a poisoned animal being used as a safe food. The present inquiry was undertaken to ascertain the exact physiological action of the root-extract upon the fish, with a view to obtaining a solution of the problem.

Two different methods have been employed in the following investigations : A, the Method of Eye Observation, and B, the Method of Automatic Record.

A.—METHOD OF EYE OBSERVATION

The results of this investigation will be given in the following order :

- (1) The application of the root-extract.
- (2) Determination of normal respiratory activity.
- (3) Effect of root-extract.
- (4) The minimally effective dose.
- (5) Comparative effect on different varieties of fish.
- (6) Non-poisonous action of the root-extract.
- (7) Contrasted effect of protoplasmic poison.
- (8) Investigation of the causes of death.
- (9) Symptoms of asphyxiation.
- (10) Inactivation of the respiratory mechanism.

I. THE APPLICATION OF THE ROOT-EXTRACT

In making the extract from the root of *Millettia pachycarpa*¹ for the following investigations, 5 grams of shavings of the root were macerated in 100 c.c. of water. The filtered solution was kept in the stock-bottle and regarded as a 5 per cent. solution. For observing the effect of different strengths of solution, the stock material was diluted to make a 1 per cent. and a 0.1 per cent. solution. The fish was placed in a vessel of water and then subjected to the action of the extract.

THE RESPIRATORY ACTIVITY

For the study of the physiological reaction induced by the root-extract, the only satisfactory method is the observation of the decline of certain visible indications of vital activity, such as the changes in the rate of respiration of the experimental fish, which is essentially a water-breathing animal, and in which the supply of oxygen and the removal of CO₂ are effected by the circulation of water, containing absorbed air, over the gills, by the periodic opening and closing of its mouth and opercula. There are fishes, locally

¹ Mr. Biren N. Ghose of Darjeeling kindly collected the particular roots.

known as Vacha (*Eutropiichthys vacha*) and Hilsa (*Clupea ilisha*), which quickly die after being taken out of water; these may therefore be regarded essentially as water-breathers. The fish *Cirrhitina reba*, can, however, live out of water for a certain length of time, though it soon begins to gasp for breath. If, after 30 minutes or so, it is replaced in water it lies on its back upside-down, apparently dead; it does not usually recover when replaced in water. *Aoria tengara*, however, can live out of water for a longer period.

Certain other fishes are known, such as *Anabas testudineus*, *Clarias batrachus*, *Saccobranchus fossilis*, and *Ophiocephalus punctatus*, which, though using their gills for respiration while in water, can remain alive outside it for a considerable length of time. In explanation of this, various theories have been offered by different observers. Some consider that the fish has reservoirs at the sides of the head which contain water for maintaining the gills in a moist condition when out of their natural element. Others think, on the contrary, that these reservoirs contain air necessary for respiration of these fishes, since they are observed to raise their mouth occasionally out of water, apparently to take in air. It is not improbable that in such cases water-breathing is supplemented by air-breathing.

This view is strongly supported by the observation of G. C. L. Howell,¹ who finds that the rise of the fish to take in air is dependent on the temperature. Thus, while the *Ophiocephalus* fish frequently rises above the water to take in air at 25·5° C., it ceases to rise when the temperature falls to 10° C. This leads him to conclude that the air-cavity is only an auxiliary apparatus to be called into use when a supply of oxygen, obtainable through the gills, is insufficient. He regards the amount of oxygen absorbed by the water from the air to vary inversely as the temperature. Hence the oxygen-content of water is less at a higher temperature, and the fish is compelled to supplement water-breathing by air-breathing.

¹ G. C. L. Howell, 'Notes on the Respiration of the Murrel (*Ophiocephalidae*),' *The Journal of the Bombay Natural History Society*, vol. xxiv, p. 195.

In regard to respiration in fishes, then, no hard and fast line can be drawn; for the two classes, water-breathers and air-and-water-breathers pass imperceptibly one into the other. The fish *Aoria* may be regarded as an intermediate case; it can remain alive outside water for several hours. In water it rises occasionally to inhale air; but when kept immersed under water by suitable means, it shows no great discomfort, and is able to live by water-breathing alone.

2. DETERMINATION OF NORMAL RESPIRATORY ACTIVITY

The root-extract will presently be shown to induce a change of respiratory activity of the fish, the normal activity being determined by counting the rate of its opercular movements (or pulsations) which maintain the circulation of water through the gills. Any modification in the respiratory activity can be found by noting the induced change in the amplitude and in the rate of the observed opercular pulsation.

Experiment 1. *Normal rate of pulsation.*—The following experiment, carried out with *Aoria*, may be taken as typical of others. This particular species offers special advantages for quantitative investigations, since :

- i. Its opercular movements are far more pronounced than those of other fishes, and can therefore be easily observed.
- ii. It usually exhibits a uniform rate of respiration which is found to be modified in a definite way, indicating a change of physiological activity induced by the specific action of various drugs. In many other fishes the respiratory activity is not, however, sufficiently regular for accurate measurement, and even a slight disturbance causes them to hold their breath for an indefinite length of time.

Returning to the normal rate of respiration of *Aoria*, it may be said that, in a condition of comparative rest, this rate is very much the same in specimens of similar size, age,

and vigour. When disturbed or frightened the fish makes a frantic rush, when the rate of its respiratory activity becomes increased as in other animals. The respiration returns to the normal rate on the cessation of the disturbing cause.

In the following experiments ten different specimens of small-sized *Aoria*, about 5 cm. long, were taken, and the number of pulsations per minute in each case determined by means of a stop-watch. The results are given in Table I.

TABLE I.—AVERAGE RATE OF RESPIRATION (*AORIA*)

Specimen	1	2	3	4	5	6	7	8	9	10
Pulsations per minute	140	140	140	133	133	133	130	130	130	130
Average rate of respiratory pulsation = 134 per minute.										

The time of a single pulsation is therefore slightly less than half a second, this being the rate of respiration of the fish when perfectly free to raise its mouth occasionally out of water. The rate of opercular pulsation in *Aoria* depends, as already stated, on its vitality as well as on the season, the variation being from about 75 to 150 per minute.

Experiment 2. *Rate of respiration of fish immersed in water.*—The experiment was carried out to find whether there is any change in the respiratory activity when the fish is kept completely immersed in water and prevented from rising to the surface. The normal rate of a particular fish when perfectly free to rise was found to be 121 per minute. The dorsal fin was then held in a clip and the fish kept under water (*cf.* fig. 5). After the disturbing effect of the brief struggle had subsided, the rate of respiration of the fish was found to be 120 per minute, which is practically the same as that of the fish when perfectly free. Being kept in this immersed position for a long time, the rate of respiration after two hours was found to have remained

practically unchanged, namely, 120 per minute. When, after this restraint, the fish was released, it swam about vigorously. Similar results were obtained with three other specimens.

3. EFFECT OF ROOT-EXTRACT

Having described the method of measurement of normal rate of respiratory activity, experiments were next carried

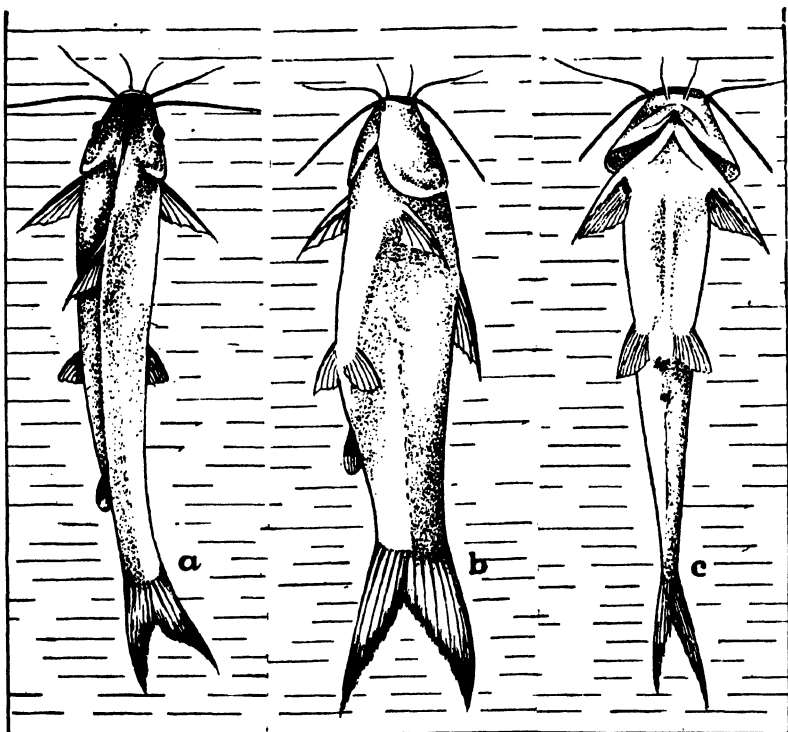


FIG. 1. Effect of root-extract on *Aoria tengara*.

- a. Normal position of the fish while swimming.
- b. Partial loss of balance ; fish lying on its side.
- c. Complete loss of balance ; fish lying upside-down.

out on the effects of different solutions of the root-extract on the rate of respiration of *Aoria*. It may here be stated

that the results thus obtained may be regarded as generally applicable to other varieties of fish, the only difference being that the effect makes its appearance earlier in some cases and later in others.

Experiment 3. *Effect of 1 per cent. solution on Aoria.*—The general effect induced may be described as follows. On application of the root-extract there is usually a preliminary stimulation, as indicated by the darting movement of the fish. After a short while it becomes relatively quiescent, and the subsequent rate of its respiratory activity is found to undergo a continuous depression. Along with this, other and more striking symptoms make their appearance. The fish becomes unsteady in its gait ; it partially loses its balance and, falling on one side, tries ineffectively to recover it ; this condition will for convenience be described as a *partial upset*. Later, the loss of balance becomes more complete, when the fish lies upside-down on its back, a condition which may be designated as one of *complete upset* (fig. 1).

The effect of the root-extract on *Aoria* depends on the size and vigour of the specimen, on individual susceptibility, as well as on the strength and duration of application of the solution. Details of the continuous diminution of the rate of respiration, as also of the time for complete upset and paralysis under the action of 1 per cent. solution, are given below for three different specimens, A, B, C.

TABLE IIA.—EFFECT OF ROOT-EXTRACT ON
RESPIRATION (*AORIA*)

Rate of respiration (normal)	140 per minute
" "	5 minutes after application	60 " "
" "	15 " "	"	"	"	"	33 " "
" "	20 " "	"	"	"	"	25 " "
" "	30 " "	"	"	"	"	Stoppage

The fish was completely paralysed and on its back upside-down after 20 minutes ; respiration came to a final stop after 30 minutes.

TABLE IIB.—EFFECT OF ROOT-EXTRACT ON
RESPIRATION (*AORIA*)

Rate of respiration (normal)		135 per minute
10 minutes after application		40 " "
25 " " "		30 " "
30 " " "		5 " "
32 " " "		Stoppage

The fish lay on its back after 25 minutes, and respiration came to a complete stop after 32 minutes.

TABLE IIC.—EFFECT OF ROOT-EXTRACT ON
RESPIRATION (*AORIA*)

Rate of respiration (normal)		145 per minute
5 minutes after application		80 " "
10 " " "		50 " "
20 " " "		10 " "
25 " " "		Stoppage

Complete paralysis occurred after 20 minutes, and arrest of respiration after 25 minutes.

Reference has already been made in regard to the belief that the death of the fish is somehow brought about by an intense irritation of the eye caused by the extract. But observations made on the subject show that the extract has no effect on the optical organ.

PROGRESSIVE EFFECT OF APPLICATION OF ROOT-EXTRACT

The following symptoms appear under continued application of the root-extract :

- (1) The fish exhibits a partial loss of balance and lies on one side.
- (2) Later, the loss of balance is more complete, when the fish becomes inverted, lying on its back upside-down.
- (3) In some instances, while thus rendered completely inert, it *floats* on its back.
- (4) In other cases, though lying on its back, it *sinks*.

The question arises: What is the probable explanation of these symptoms? It may be thought that the adjusting mechanism, by which the fish maintains its normal position, controlled by its muscular power, is disturbed by the action of the root-extract. The adjusting organ may either be the lateral or terminal fins, or it may be the swim-bladder.

Assuming that the fins are essential in maintaining the normal position, the loss of muscular power, by the action of vegetable extract, would induce their inactivation. The heavy back of the fish would then sink, whereas the belly, in which is the swim-bladder containing air, would rise to the surface like a balloon.

This explanation cannot, however, be regarded as fully satisfactory, since after a short application of the root-extract the fish is not completely upset, *i.e.* upside-down, but lies on one side. The equal inactivation of the opposite pairs of fins cannot, therefore, explain this particular attitude of the fish. The inactivation of the fins could only transform the normal swimming position of the fish into one of upside-down.

The following experiments were undertaken to obtain additional knowledge of the intricacies of the problem in regard to the relative effectiveness of the fins or of the swim-bladder in maintenance of the normal swimming position.

Experiment 4. *Effect of removal of the fins.*—The side and tail fins were cut off; the fish, nevertheless, maintained its normal position. When turned over, the fish immediately regained the normal position, the maintenance of which is not, therefore, dependent on the activation of the fins.

The fish, deprived of its fins, was next subjected to the action of the vegetable extract. It then exhibited, in proper sequence, all the characteristic effects shown by the intact fish; that is to say, a short application caused the fish without fins to lie on its side, while a longer application caused the fish to turn upside-down.

Since the fins are not essential for the purpose of adjustment, it may be thought that the swim-bladder is at least

partially effective for the purpose. This supposition receives some support from the following observations.

Experiment 5. *The swim-bladder as an adjusting organ.*—The fish under prolonged action of the root-extract becomes inert and, as already stated, lies in a passive condition upside-down. Some of the fishes float on the surface of the water, their bright under-side rendering them conspicuous whilst being carried down the stream. In other cases instead of floating they sank under water. What could be the cause of this difference?

In answer to this question, dissections were made of the fishes which were found floating, and also of those which had sunk under water. In the first case the air-bladder was found to be tense, being inflated with air; this gave the fish sufficient buoyancy for floating. In the second case the air-bladder had collapsed and was comparatively empty of air. It appeared that in such a case the fish, during its struggle which preceded death, had ejected air from the bladder and, being thus deprived of buoyancy, sank under water. This inference is verified by the following observations carried out with a number of fish which had been floating. The fish was held under water and subjected to squeezing, when the escaping air from the swim-bladder through the mouth was seen bubbling through the water. As a result of this, the previously floating fish sank immediately.

4. THE MINIMALLY EFFECTIVE DOSE

The strength of solution employed in the experiments summarised in Table II was 1 per cent. The following results prove that an excessively minute dose may even be effective in causing the death of the fish.

Experiment 6. *Effect of a minute dose.*—Five small *Aoria* fish were placed in a shallow round dish, 25 cm. in diameter, containing 1000 c.c. of water. The creatures were actively swimming about, the rate of their respiration being normal. A piece cut from the *Millettia* root was now placed in the centre of the dish, when the active

substance, slowly dissolved by the water, became gradually diffused. The fishes became greatly disturbed and were completely upset in the course of less than half an hour.

The extract of the root of *Millettia* is thus seen to be very effective in inducing inertness, facilitating the capture of the fish.

In the next experiment a definite dilution of the extract was employed.

Experiment 7. *Effect of a dilute solution, one in a thousand.*—A large-sized *Aoria* was found to have the rate of respiration of 120 per minute. When placed in the dilute solution its respiratory activity became continuously slowed down. It lost its balance in the course of 12 minutes, when its opercular pulsations were reduced to 87 per minute. The paralysis was complete, and respiration came to a final stop after 40 minutes.

5. COMPARATIVE EFFECT ON DIFFERENT VARIETIES OF FISH

The root-extract is found to exert similar action on all varieties of fish, rendering them inert and moribund for easy capture. The results given in the following table may be taken as typical; those which cannot live out of water for any great length of time are placed at the beginning of the list, and those which can remain alive for relatively longer periods, towards the end.

TABLE III.—EFFECT OF ROOT-EXTRACT ON DIVERSE SPECIES OF FISH

Specimen.	Time for partial loss of balance	Time for complete paralysis
<i>Cirrhitina reba</i> . . .	3 minutes	10 minutes
<i>Aoria tengara</i> . . .	5 "	20 "
<i>Trichogaster fasciatus</i> .	6 "	25 "
<i>Ophiocephalus punctatus</i>	16 "	30 "
<i>Ophiocephalus striatus</i> .	12 "	28 "
<i>Clarias batrachys</i> . .	17 "	37 "
<i>Anabas testudineus</i> .	28 "	40 "

Observations were made of the action of 1 per cent. solution of the extract. The species of the fish is indicated in the first column of Table III, the time required for loss of balance is given in the second, while the period necessary for complete paralysis, causing the fish to turn upside-down, is given in the third column.

6. NON-POISONOUS ACTION OF THE ROOT-EXTRACT

The question whether the root-extract acts as a general protoplasmic poison will next be subjected to the following experimental tests :

- i. A poisoned animal cannot be consumed without fatal results.
- ii. Direct application of a poisonous solution on different organs of an animal must necessarily abolish their characteristic functional activity.
- iii. It would be impossible to revive an animal which had been subjected to a general protoplasmic poison ; whereas an animal under local paralysis, induced by a drug, could be revived after suitable treatment.

Experiment 8. *The harmlessness of the killed fish as food.*—A number of fish which died after prolonged application of the root-extract were exposed in the open, when the crows consumed them with great avidity. The birds did not experience any evil effect whatsoever. Fishes killed by the extract, as already stated, are also safely consumed by human beings.

Experiment 9. *Effect of injection into the stomach of the fish.*—Three c.c. of strong solution of the root-extract were introduced into the stomach of an *Ophiocephalus* by means of a syringe ; the fish was then replaced in a vessel of fresh water. The treatment did not cause any harm, as the fish swam about freely and remained fully alive. The extract introduced into the stomach does not, therefore, exert any poisonous action on the fish as a whole.

Experiment 10. *Effect of injection on Guinea-pig.*—

A fairly large quantity of the strong stock solution was forced into the stomach of a guinea-pig. The creature was in no way inconvenienced by this treatment, but continued for months to enjoy its normal health.

Experiment 11. *Persistence of cardiac activity in a moribund fish.*—The experiment was carried out with *Ophiocephalus striatus*. It was subjected to the action of a 1 per cent. solution of the root-extract, under which it became moribund and turned upside-down. After a short time its respiratory activity also came to a stop.

The fish, which was apparently dead, was then opened ; its heart, nevertheless, exhibited active pulsation which persisted unchanged for several hours. This leads to the conclusion that the solution causes a paralysis only of the respiratory process, without affecting the cardiac activity. A more rigorous demonstration of this will be given later.

Experiment 12. *Revival of the moribund fish by method of artificial respiration.*—This experiment was carried out with *Aoria*, the rate of its normal respiration being 120 per minute. When placed in a dilute solution of the root-extract, the rate of respiration became reduced to 100 per minute in the course of 10 minutes ; after 15 minutes it became further reduced to 50 per minute, culminating in an arrest after 25 minutes. The fish was by this time completely paralysed and lay on its back upside-down.

Attempts were then made to revive the fish by the method of artificial respiration. It was placed in a vessel of water through which a stream of oxygen was kept bubbling by the opening of a stop-cock connected with a gas cylinder. The opercular pulsation, indicating respiratory activity, was now feebly renewed ; the fish, however, still remained in a passive condition on its back and would have succumbed but for the enforced artificial respiration produced by repeatedly opening and closing the operculum. This method of enforced respiration is not unlike that adopted for reviving a drowned human being.

In a typical case of the effect of this artificial respiration, the rate of the revived opercular pulsation became 100 per minute after 45 minutes. The fish now turned over and

assumed its normal position, swimming about with perfect freedom and vigour. Similar results were obtained with three other specimens.

But in cases where the respiration had been arrested for too long a period by the action of the root-extract, attempts at revival by artificial respiration failed. It is true that there was, even in such cases, a feeble revival of the respiration; this was, however, found to be temporary, since the fish succumbed ultimately. Failure of revival in such cases is evidently due to excessive accumulation of CO_2 in the blood which could not effectively be eliminated by artificial respiration.

7. CONTRASTED EFFECT OF A PROTOPLASMIC POISON

The characteristic effect of a protoplasmic poison on an animal is that it abolishes the activity, not merely of one, but of all its organs. This is exemplified by the action of potassium cyanide.

Experiment 13. *Effect of dilute solution of Potassium Cyanide on a swimming fish.*—Potassium cyanide is an extremely poisonous substance. A vigorous *Aoria* fish was actively swimming in a vessel of water. When some KCN solution was added to the water, so as to make the solution as dilute as one part in a thousand, the fish became greatly disturbed. This was followed by a complete paralysis and the fish lay on its back in the course of 3 minutes. The respiration, as indicated by the opercular movements, also came to a complete stop. After allowing the fish to remain in the poisonous solution for another 5 minutes, it was replaced in fresh water through which oxygen was kept bubbling. But all attempts to revive it by artificial respiration failed altogether.

Experiment 14. *Effect of introducing poison into the stomach.*—Strong solution of KCN was introduced into the stomach of a very vigorous *Ophiocephalus* fish by means of a syringe. It was then placed in a vessel of water. The fish turned upside-down in less than 2 minutes. Subsequent examination showed that the fish was dead without

any possibility of revival. It is to be remembered, in this connection, that a similar introduction of the vegetable extract did not produce any evil effect on the fish (*cf.* Experiment 9).

Experiment 15. *Stoppage of the heart-beat.*—When the fish was placed in KCN solution, the poison became diffused and soon affected its internal organs. In an experiment with a specimen of *Aoria* placed in dilute KCN solution the respiratory activity came to a stop in about 3 minutes. After keeping the fish in the solution for a further period of 10 minutes, it was opened for examination. The cardiac activity had by that time been completely abolished.

Experiment 16. *Effect of direct application of KCN solution on the heart.*—A vigorous specimen of *Aoria* was opened and the rate of its cardiac pulsation was found to be 75 per minute. Direct application of dilute solution of KCN on the heart brought the pulsations to a state of stand-still in the course of 2 minutes. A similar experiment was carried out with *Anabas*. The direct application of the KCN solution on the heart caused an immediate slowing down of the rate of the heart-beat. The pause between the successive beats became longer and longer, and the heart came to a permanent stop after 3 minutes. It will be shown later that direct application of the root-extract does not affect the cardiac activity (*cf.* Experiment 33). How different is the effect of the root-extract to that of protoplasmic poison will be demonstrated later in Experiments 53 and 54.

The experiments described above prove :

- i. That the fish which died under *prolonged* application of the root-extract can be eaten without any harm.
- ii. That the root-extract proves to be perfectly harmless when introduced into the stomach of the fish or of the guinea-pig.
- iii. That, though the root-extract causes an arrest of respiratory activity, it does not affect the cardiac activity of the fish.
- iv. That the breathing of the fish, arrested by the action

of the root-extract, can afterwards be revived by the method of artificial respiration.

- v. That the effect of a protoplasmic poison on the fish is essentially different, since all vital activities are abolished without any possibility of revival.

These results offer conclusive proof that the fish is not poisoned by the root-extract.

8. INVESTIGATIONS ON THE CAUSE OF DEATH

In what way then does the root-extract, without being poisonous, cause the death of the fish? Are there any ways, other than the application of the root-extract, which induce parallel effects, such as the loss of balance followed by complete paralysis, with the possibility of revival by artificial respiration? If so, then a common underlying factor may be found for discovering the particular physiological mechanism, the inactivation of which causes death.

The results of experiments already described prove that the root-extract brings about a continuous diminution and final arrest of respiratory activity. It would thus appear that death is probably due to arrest of respiration, *i.e.* asphyxiation. The correctness of this inference can be tested by observing the effect induced by either a partial or a more complete asphyxiation, brought about by widely different means.

9. SYMPTOMS OF ASPHYXIATION

For the maintenance of the life of the fish it is essential that the respiration should be sufficiently active for the exchange of the carbonic acid in the blood for the oxygen in the water. This is, as already stated, ensured by the circulation of water containing dissolved air through the gills, by alternate opening and closing of the mouth and the opercula. The fish will become asphyxiated: (1) when the normal process of respiration is interfered with; (2) when the water-breathing fish is kept out of water for a long time; and

(3) when the water for respiration is deprived of the air which contains the oxygen. The normal respiration may be interfered with in the following ways :

Experiment 17. *Effect of enforced gaping.*—The fish *Aoria* was prevented from closing its mouth by vertically inserting a piece of match-wood, the mouth being thus kept widely open. After this the opercular movements of the fish still persisted, though greatly enfeebled ; for the respiration could not be as effective as when the opening and closing of the mouth also helped in the process. Under enforced gaping the fish exhibited increasing distress ; it lost its balance and then lay upside-down in the course of 5 minutes, the symptoms being exactly parallel to those of a fish rendered inert by the root-extract.

The asphyxiation produced by enforced gaping is, for reasons already given, only partial. Hence removal of the vertical piece of match-wood might be expected to bring about recovery without much difficulty. As a matter of fact, the respiratory activity, after this procedure, became fully restored ; the fish turned over and, regaining its normal balance, swam about with its accustomed vigour.

Experiment 18. *Effect of enforced closure of mouth and of opercula.*—The respiratory process was more seriously interfered with by tying a thin string round the opercula and also sewing up the mouth. The normal rate of respiration of a vigorous *Aoria* was, before this operation, 140 per minute. After the enforced closure of the mouth and opercula the fish was replaced in water, when for a time it rushed about in great distress ; it then lost its balance and was completely upset in the course of 8 minutes. Ten minutes later the mouth and the opercula were freed from the restraining string. After this prolonged asphyxiation, the fish, which lay on its back gasping for breath, did not succeed in recovering its balance. Revival was only effected by the special treatment already referred to—that is to say, by artificial respiration ; the fish now became fully revived, when it turned over and swam about with its normal vigour.

Other modes of producing asphyxiation are described below.

Experiment 19. *Effect of removal from water.*—The normal respiration by the gills is obviously interfered with when the fish is taken out of the water. For the following experiment the fish *Cirrhina* was employed. When taken out of water it began to gasp for breath; after having been kept outside its proper element for half an hour or more it was replaced in water. The usual symptoms of asphyxiation were now exhibited, the fish lying upside-down.

Revival by enforced respiration.—In cases of excessive or prolonged asphyxiation, attempts at resuscitation proved to be futile. But when the asphyxiation had only been moderate then the method of artificial respiration was found to be effective in ensuring complete recovery.

Experiment 20. *Asphyxiation by excessive CO₂ in water.*—In the cases already described the asphyxiation was produced by a more or less effective interference with the normal process of respiration through the gills. In the present case a more intense asphyxiation was produced by placing the fish in water which did not contain air or oxygen, but charged with an excess of CO₂. On placing a dozen vigorous *Aoria* fish in soda-water, which was overcharged with this gas, they all became completely upset and moribund in a time as short as 3 minutes. The induced asphyxiation was so great that artificial respiration failed to revive the fish.

While the root-extract does not cause any poisonous action, all the symptoms induced by it are precisely the same as those of direct or indirect asphyxiation; the successful method of ensuring recovery is also the same in the two cases.

These experiments lead to the conclusion that the root-extract causes the death of the fish by asphyxiation.

The investigation must now be carried one step further for obtaining some insight into the manner in which the respiratory mechanism becomes inactivated, bringing about asphyxiation and death.

10. INACTIVATION OF THE RESPIRATORY MECHANISM

Since the root-extract does not exert any effect on the alimentary canal, nor on the heart, it would seem that it induces only a local paralysis of the respiratory process, which is actively maintained in the fish by the opercular pulsations. The effect induced by the extract may therefore be described as being due to local paralysis of the respiratory organ. In this connection it would be interesting to study the effect of the well-known anæsthetic agent ether on respiration.

Experiment 21. *Paralysing action of Ether*.—Two different experiments were carried out with *Aoria*. In the first case the rate of normal opercular pulsation in water was found to be 130 per minute. The fish was then transferred to a vessel of water to which ether had been added, so as to make a dilute solution of 0.5 per cent. The effect of the anæsthetic was a continuous decline of the respiratory activity, attended sometimes by a preliminary enhancement. The continuous decline in the rate of respiration resulted in a complete paralysis of the fish, which lay on its back in the course of a few minutes. As regards the respiratory activity, the frequency of opercular pulsation was reduced from the normal 130 to 60 per minute in the course of 3 minutes; after 4 minutes it became further reduced to 30 per minute, and was completely arrested after 7 minutes.

The results of application of 0.5 per cent. ether are given in the following table.

TABLE IV.—RESPIRATORY MECHANISM INACTIVATED BY ETHER (*AORIA*)

Normal rate in <i>Aoria</i>	130 per minute
Immediate stimulatory effect	150 „ „
After 3 minutes	60 „ „
„ 4 „	30 „ „
„ 7 „	Arrest

The fish lay on its back after 7 minutes.

The induced paralysis was, however, found to be temporary, for when replaced in fresh water the fish revived, turned over, and swam about with perfectly restored vigour.

Experiment 22. *Local paralysis under Ether*.—In order to prove that the ether had only induced a local paralysis of the respiratory organ, the fish was opened for examination of its cardiac activity. In spite of the fact that the respiration of the fish had come to a stop, the heart-beat was still found to be active, the rate being 65 per minute. In a similar specimen which had not been treated with ether the rate of the cardiac pulsation was 70 per minute. In a previous experiment (Experiment 11), the root-extract has already been shown to induce local paralysis of respiratory activity without affecting the cardiac pulsations.

The various facts described lead to the conclusion *that the root-extract does not affect the fish as a whole, but only causes a local paralysis of its respiratory organ.*

B.—THE AUTOMATIC METHOD OF RECORD

The method of eye-observation has the advantage of simplicity, since the only apparatus required is a stop watch for determination of the frequency of pulsation. But for advanced investigations on the subject it is not sufficiently accurate for the following reasons :

- i. The respiratory efficiency is determined not merely by the frequency but also by the amplitude of the periodic opening and closing movements of the opercula and of the mouth. It is, however, impossible by mere eye-observation to measure the induced variation of the amplitude.
- ii. The frequency of pulsation, as determined by eye-observation, is calculated as the average of results obtained after prolonged observation. This frequency may not have remained constant but undergone a fluctuation.
- iii. Personal error cannot be strictly excluded when the time to be measured is as short as half a second or less.

For the clear understanding of the process of the respiration of the fish and any variation induced in it, it is necessary to obtain exact knowledge of the working of the respiratory mechanism by detecting the minutest change in the rate throughout a long series of successive cycles.

This is only possible by the invention and perfection of a device for automatic and continuous record of the effect of external variations on the respiratory activity of the fish, as effected by the periodic movements either of the operculum or of the mouth. A description is given below of the successful methods which I have been able to devise for the automatic records on a moving plate of smoked glass of the opercular pulsation as well as of the pulsation of the mouth.

AUTOMATIC METHOD IN THEORY AND PRACTICE

For recording the opercular pulsation, the fish has to be held sideways on a wooden base with one of its opercula facing upwards. A portion of the wood is properly scooped out so that the fish may lie comfortably on its side, care being taken that the lower operculum is also perfectly free to execute its opening and closing movements. A surgical bandage ties the fish to the wooden base so that the creature is made to lie still; the bandage should not be too tight so as to interfere with the freedom of its respiration. The periodic movements of the upper operculum is recorded by means of a recording lever, the short arm of which is connected with the operculum by a thin thread. This thread, passed through the operculum, has a knot at the lower end to prevent it from slipping, the connection being made at a maximum distance from the hinge of the operculum, so that the extent of the movements of opening and closing may be as large as possible. An additional enlargement of about 3 times is produced in the curve by the magnifying writing lever, the amplitude of recorded pulsation being then about 2 cm. This can, however, be increased still further to about 5 cm. The record of pulsations is taken when the fish is placed in water or in any other liquid.

THE RESONANT RESPIROGRAPH

The smoked-glass plate for the record is made to move laterally at a rate of about 3 mm. per second by means of a clockwork. An error in the exact time-relation of the recorded curve of respiration arises from the friction of

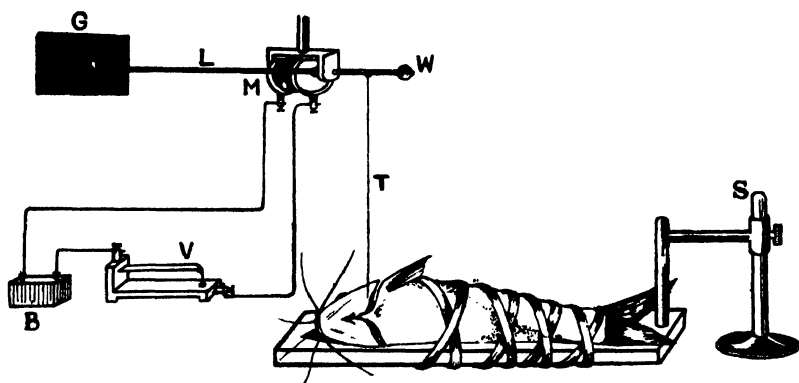


FIG. 2. Method of recording opercular pulsation by Resonant Spirograph.

Fish tied sideways by surgical bandage on wooden base; s, adjustable stand for keeping fish under water. Thin thread *t* passed through operculum, attached to short arm of writing lever which is nearly balanced by counterpoise *w*. The steel writer of the Resonant Recorder is supported at centre of an electromagnet *M*, the magnetising coil of which is in circuit with storage cell *B* and vibrating reed *V* for periodic completion of electric circuit.

contact of the tip of the writing lever against the surface of the smoked glass. The source of this error has been eliminated by the Resonant Spirograph, specially devised for this and similar purposes, in which the tip of the writing lever is not in contact with the smoked-glass plate, but at a short distance in front of it. The writer is a fine steel wire which is maintained in a state of resonant vibration, being exactly tuned to vibrate, say, twenty times in a second; it is supported on jewel bearings at the centre of one pole of an electromagnet. The magnetising coil is in circuit with a storage cell *B* and a vibrating reed *V*, which periodically completes the electric circuit (fig. 2). When the reed is

exactly tuned to vibrate 20 times in a second, the writing lever is thrown into sympathetic vibration and strikes the smoked-glass plate once in every twentieth of a second. With finer recorders it is possible to measure time-intervals as short as a hundredth or a thousandth of a second. When the writer vibrates twenty times in a second, the successive dots in the record measure time-intervals of 0.05 second. The advantage of a dotted record is (1) that the error arising from friction is removed, and (2) that the most accurate time-measurement of different phases of the curve of respiration is obtained from the dots inscribed in the recorded curve itself. The period of closure movement, the duration of the intervening pause, as well as the characteristic variations of these under external agencies, can thus be determined in a quantitative manner and with an unprecedented accuracy.

METHOD OF EXPERIMENT

The wooden base which holds the fish on its side is, as already explained, supported by an adjustable stand; the fish is then immersed in a rectangular trough 15 cm. in length, 8 cm. in breadth, and 9 cm. in height, the capacity of the trough being about a litre. The vessel may be of glass or of nickel-plated metal. The inner surface of the vessel is thickly coated with solid paraffin, so that traces of chemical agents used for one experiment may be thoroughly washed off before the commencement of the next.

Several series of records can be taken on the same plate by successively raising it about 2.5 cm. at a time, the plate being held securely in each position by means of side-springs. The experimental procedure is as follows: the record of normal opercular pulsation in fresh water is first taken; the water is then allowed to run out of the vessel by removing the india-rubber cork which closes the large exit pipe. The required chemical solution is then poured in to replace the water, the exit pipe being now closed. This procedure avoids all unnecessary disturbance, and the pure effect of the particular solution can be recorded within a very short time. For studying the influence of variation of

the dose, different strengths of solution are applied, such as 0.1 or 1 per cent.

The complete apparatus is shown in fig. 3, reduced to one-fifth actual size. The resonant writer is thrown into sympathetic vibration by the vibrating reed. The fish, not shown in the figure, is securely bandaged and immersed in water, or in any required chemical solution, in the trough T. The operculum is attached to the short arm of the writing

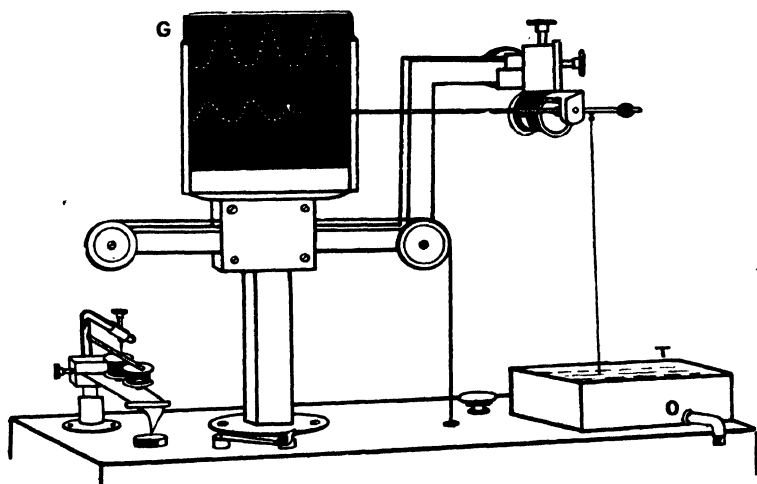


FIG. 3. Complete apparatus for recording the respiratory activity of the fish.

T, trough for holding water or other chemical solution (fish not shown); O, outlet pipe. The smoked-glass plate G can be raised 2.5 cm. at a time for successive records.

lever. The complete instrument is very compact and portable, and is not put out of working order even after a rough and long journey. A complete series of experiments can be carried out in less than an hour, during which time the fish has to be kept completely immersed.

The question now arises :

- i. Whether the normal rate of respiration of the fish, completely immersed in water, remains the same as when it is free to rise occasionally to the surface.

- ii. Whether the mechanical restraint, imposed by holding the fish on one side by the bandage, interferes with its natural respiratory activity.

In regard to the first of these questions it has been shown (*cf.* Experiment 2) that the rate of normal respiration of *Aoria*, as indicated by the frequency of opercular pulsation, remains the same for several hours whether the fish is free to rise or is kept immersed in water. This is equally true of the opercular pulsation of certain other fishes such as *Cirrhitina*.

In regard to the second question, one cannot but have a misgiving that the natural rate of respiration is likely to be modified when the fish is held on one side under the restraint of a bandage. It was therefore a matter of considerable surprise to find that this restraint did not produce any noticeable change in the normal rate of respiration. It is true that the struggle made by the fish on being tied up caused at first an irregularity in the rate. But this disappeared in the course of 10 minutes or so, by which time the fish had become accustomed to the new situation.

The following experiment demonstrates the very remarkable uniformity of pulsation which is maintained for a considerable length of time. The only external variation which is likely to affect the normal activity is the change of temperature. But in the experimental room at midday the temperature of the water in the vessel remains practically constant for a couple of hours at least.

I will first explain the experimental method of opercular pulsation, that of the mouth being described later.

RECORD OF OPERCULAR PULSATIONS

Experiment 23. *Uniformity of normal rate of respiration.*—A vigorous *Aoria* fish was taken for the two following series of experiments. When perfectly free its rate of opercular pulsation was carefully determined by a stop-watch, and found to be 150 per minute, the average rate of each complete pulsation being 0.40 second.

The fish was then mounted in the manner already de-

scribed, and two series of records taken, the first after a short period of rest and the second after a further interval of two hours. A comparison of the two would show whether the respiratory activity remained constant during that period or had undergone a variation.

By simply counting the total number of dots in each curve, it is possible to determine with unprecedented accuracy the period of each complete pulsation. The curve



FIG. 4. Records showing constancy of rate of opercular pulsation (*Aoria*).

a. Taken at the beginning.

b. Taken after 2 hours.

In record of opercular pulsation the up-curve represents movement of closure, down-curve that of opening.

Successive dots in all records at intervals of 0.05 second.

of each pulsation, say the first in the series, shows that there are 8 dot-intervals, each of which represents 0.05 second. Hence the period of a complete pulsation is $8 \times 0.05 = 0.40$ second. This, it will be remembered, is also the average value calculated from eye-observation when the fish was in a state of perfect freedom. Still more remarkable is the maintenance of this constancy for at least two hours, as indicated by the series given in the second row (fig. 4). This constancy can be secured by choosing sufficiently good specimens. The following table gives the time

of complete pulsation for each one of the series of five, recorded at the beginning and then at the end of two hours.

TABLE V.—TIME, IN FRACTIONS OF A SECOND, OF EACH OF FIVE SUCCESSIVE PULSATIONS (*AORIA*)

Number	First	Second	Third	Fourth	Fifth
At the beginning .	0·4 sec.	0·4 sec.	0·4 sec.	0·4 sec.	0·4 sec.
After two hours .	0·4 „	0·4 „	0·4 „	0·4 „	0·4 „

RECORD OF PERIODIC OPENING AND CLOSING MOVEMENTS OF THE MOUTH

A very different method of determining the frequency of respiration is also afforded by automatic record of the periodic movements of the mouth. It has been explained that the circulation of water through the gills for respiration is brought about by the opening and closing movements of the opercula as well as that of the mouth. The method of recording opercular pulsation has already been described ; that of the periodic movements of the mouth is given below.

The upper jaw of the fish is stationary, whereas the lower jaw plays vertically up and down alternately, and it is the movements of the latter that have to be recorded. In order to secure this, the fish is suitably held in the normal position, and not sideways as in the previous experiments. When the dorsal fin is held by a clip and the fish kept slightly pressed against the wooden base, it remains fairly quiet, unless it is exceptionally large and vigorous ; a bandage may also be employed as an additional security. The next difficulty encountered is that the lower jaw does not project out sufficiently beyond the upper ; hence the thread which connects the lower jaw to the recording lever is obstructed by the upper one. This drawback is overcome by sewing a short length of thin German-silver wire to the underside of the lower jaw, beyond which it projects by about 5 mm. The hook at the free end is attached by a thin thread to the short arm of the recording lever (fig. 5).

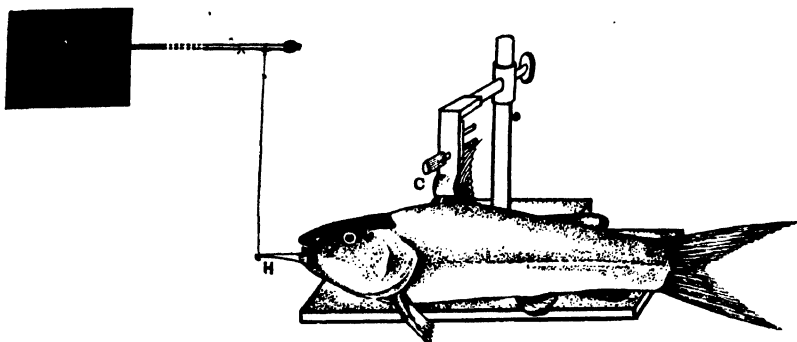


FIG. 5. Method of recording the pulsation of the mouth. Fish kept under water pressed against the base by a clip c. Short length of German-silver wire attached to lower jaw; a thin string connects the hooked free end H with short arm of recording lever.



FIG. 6. Records showing constancy of rate of pulsation of the mouth (*Aoria*).

a. Taken at the beginning.

b. Taken 2 hours later.

Note that in the record of pulsation of the mouth (in contrast with that of opercular pulsation) the up-curve represents movement of opening, the down-curve that of closure.

Experiment 24. *Record of pulsation of the mouth.*—Two series of records of the periodic movements, taken at an interval of 2 hours and reproduced in fig. 6, show the maintenance of remarkable constancy. The particular specimen of *Aoria* was moderately active, and each pulsation was completed after 11 dot-intervals. The period of a single pulsation was therefore, 0.55 second, the frequency being 109 per minute. This was also the frequency after the expiration of two hours. The records of the periodic opening and closing movements of the mouth are thus found to be essentially similar to those of the opercular pulsation.

The very striking demonstration of the constancy of the normal rate of respiration by two entirely different methods would justify the conclusion that any change induced in it must be ascribed to the effect of some variation of environmental condition, physical or chemical.

CHARACTERISTICS OF THE RECORDED CURVE

Having described two different methods of recording respiratory activity, the next question to be considered is the extent of the modification in the time-relations of the up- and down-curves, which might be produced by the tension exerted by the writing lever. In practice it is necessary that the lever, which records the pulsations, should exert a certain amount of upward tension ; otherwise it becomes too unstable for satisfactory record. The weight of the longer arm of the lever causes a marked upward tension. This can be reduced to a minimum by adding a small ball of wax as the counterpoise. By addition or removal of a portion of the wax the tension can be rendered feeble or more pronounced. The variations induced by change of tension on the records of opercular pulsation and of the pulsation of the mouth are more or less similar. For purpose of simplicity detailed account will be given of the effect of change of tension on the record of opercular pulsation.

Experiment 25. *Record under minimal tension.*—The records of opercular pulsation are at first taken under

minimal tension. The particular *Aoria* with which the experiment was carried out completed a single pulsation in 0.45 second, the frequency being 133 per minute. The record (fig. 7, *a*) exhibited a difference as regards the time taken for the operculum to complete its closing movement

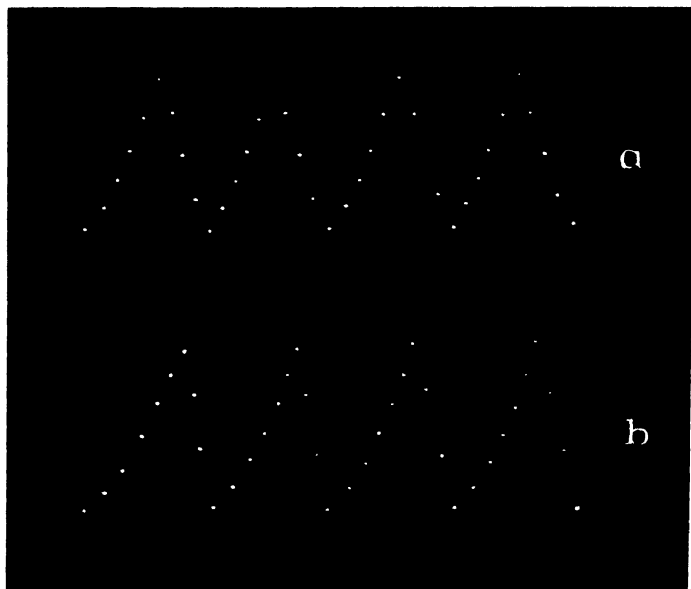


FIG. 7. Effect of variation of tension on time relation of the recorded curve of opercular pulsation (*Aoria*).

a. Record under feeble tension.

b. Record under increased tension.

Note that increase of upward tension, opposing the movement of closure, retards its rate (up-curve). The tension helping movement of opening causes acceleration of that movement (down-curve). The total period for complete pulsation remains unchanged.

(up-curve) and its opening movement (down-curve). It will be noted that the closing movement was completed at the 5th dot, *i.e.* after $5 \times 0.05 = 0.25$ second. The opening movement was, however, executed in a shorter time, after the 4th dot, *i.e.* after $4 \times 0.05 = 0.20$ second. The total time for a complete pulsation, as deduced from the dotted curve, is $5 + 4$ dots, or $9 \times 0.05 = 0.45$ second.

Further consideration tended to show that this unequal rate in the recorded up- and down-curves is, to a certain extent, due to the different conditions of recording the two movements. This will be understood from the fact that the movement¹¹ of closure (up-curve) had to be executed against the tension exerted by the recording lever, which would therefore somewhat retard the rate. The movement of opening, on the other hand, helped as it is by the upward tension, would accelerate the rate to a similar degree. Under the ideal condition of record without any tension the difference between the rates of the two movements would be lessened.

Record under increased tension.—The above inference is fully supported by the following experiment, carried out with the same specimen. If the elimination of tension tended to reduce the difference between the rates of the movements, then an increase of tension would accentuate the difference. The tension was accordingly increased by removing a portion of the weight from the counterpoise; the closing movement of the operculum was now found to have become still slower, being complete at the 6th dot, in the place of the 5th dot as in the previous case.

The opening movement was, on the other hand, accelerated by the same amount and was completed earlier, namely, at the 3rd dot (fig. 7, *b*).

The results are summarised below.

TABLE VI.—EFFECT OF VARIATION OF TENSION ON THE RATE OF CLOSING AND OPENING OF THE OPERCULUM (*AORIA*)

Tension	Period of closing	Period of opening	Total period for complete pulsation
Feeble tension	$5 \times 0.05 = 0.25 \text{ sec.}$	$4 \times 0.05 = 0.2 \text{ sec.}$	0.45 sec.
Considerable tension	$6 \times 0.05 = 0.30 \text{ ,,}$	$3 \times 0.05 = 0.15 \text{ ,,}$	0.45 ,,

The above results prove :

- i. That the closing movement of the operculum is retarded by the upward tension exerted by the recording lever.
- ii. That the movement of opening of the operculum is hastened by the tension.
- iii. That the difference between the two rates becomes lessened with the diminution of tension.
- iv. That the total time of a single pulsation given by the record is practically unaffected by a moderate variation of tension exerted by the recording lever.

It should be stated here that in certain specimens the closing movement of the operculum is very active, so that in spite of the opposing tension its recorded rate is quicker. Again, the effect of certain external variation may cause a quickening of the rate of closing.

Having secured the conditions for obtaining a perfect record, and having also proved that the normal rate of opercular pulsation remains very constant for a considerable length of time, it is now possible to study the effect of change of environment from the corresponding variation in the record of the respiratory activity of the fish.

The following results relate to the effects of change of physical environment, such as variation of temperature. The effects of root-extract and of various anæsthetics, depressants, and poisons, will also be studied in regard to their characteristic reactions in modification or abolition of the respiratory activity.

In the following investigations the changes induced in the respiratory activity will be studied by the two independent methods, that of the record of *Opercular Pulsation*, and that of the record of the periodic movements of the jaw, which for simplicity will be described as the *Pulsation of the Mouth*. Certain differences in the recorded curves obtained by the two different methods should, however, be borne in mind. In the record of opercular pulsation the up-curve represents the movement of closure, while in that of the pulsation of the mouth the up-curve indicates the

movement of opening. Again, the down-curve in the first case represents the movement of opening of the operculum, in the second case the down-curve represents the closure movement of the mouth.

The muscles that activate the operculum and the jaw are not the same ; hence the induced modifications recorded by the two different methods cannot be expected to be identical, though they are likely to be of a parallel character. The individuality of the specimen has also to be taken into account, some fish being more resistant to adverse circumstances than others. The particular examples illustrative of the characteristic effects exhibited by the records may be taken as typical, being what occurs in the generality of cases.

The investigations by the method of automatic record of Opercular Pulsation and of Pulsation of the Mouth will be described in the following order :

- (1) Effect of variation of temperature.
- (2) Effect of root-extract.
 - (a) Action of a feeble dose.
 - (b) Action of a strong dose.
- (3) Effect of the root-extract on the cardiac activity.
- (4) Effect of carbonic acid.
 - (a) Action of dilute CO_2 .
 - (b) Action of strong CO_2 .
- (5) Effect of the mild anæsthetic, ether.
- (6) Effect of the strong anæsthetic, chloroform.
- (7) Effect of coal gas.
- (8) Effect of sulphuretted hydrogen.
- (9) Effect of carbon disulphide solution.
- (10) Effect of potassium bromide solution.
- (11) Effect of copper sulphate solution.
- (12) Effect of solution of potassium cyanide.
- (13) Effect of injection of solution of root-extract.
- (14) Effect of injection of protoplasmic poison.

I. EFFECT OF VARIATION OF TEMPERATURE

The minimum temperature in the winter months in Bengal may be as low as 12° C. *Aoria* and some other fishes then fall into a state of hibernation, when they bury themselves in the mud.

OPERCULAR PULSATION.

The two following experiments were carried out with two specimens of *Aoria*, one of which was in a feeble and the other in a more vigorous condition. The mode of procedure is first to take record of the normal rate of respiration in water at the temperature of the room. The effects of cold and heat are then recorded by successively pouring cold or warm water into the trough which contains the fish. A sudden change of temperature, either a fall or a rise, causes an irregularity in the rate of pulsation, since a sudden thermal variation acts as a shock. But in the course of a few minutes the fish becomes accustomed to the new condition, and it is then possible to record the characteristic reaction to change of temperature.

Experiment 26. *Effect on a feeble specimen.*—A series of records were taken first of the normal rate, the water being at the temperature of the room, 21.1° C. The temperature of the water was then lowered to 12.2° C., and finally it was raised to 28.3° C. Each opercular pulsation at 21.1° C. was completed in 0.85 seconds, the frequency of respiration being 71 per minute. Lowering of temperature to 12.2° C. caused a marked fall of amplitude of the pulsation, leading ultimately to its arrest. This arrest was, however, not permanent, for when the temperature was raised to 28.3° C. the pulsations were not merely revived, but the amplitude and frequency became enhanced. The period of a single pulsation was thus shortened from 0.85 at 21.1° C. to 0.6 second at 28.3° C., the increase of frequency being from 71 to 100 per minute. A very characteristic variation exhibited by this record at the higher temperature is that the movement of opening of the

operculum (down-curve) was not continuous, but occurred in two sub-pulses (fig. 8).

Experiment 27. *Effect on a vigorous specimen.*—The records in this experiment were taken throughout a complete cycle of change of temperature in four stages; first, when the temperature of the room and of the water was 22.2°C .; second, when it was lowered to 12.2°C .; third,

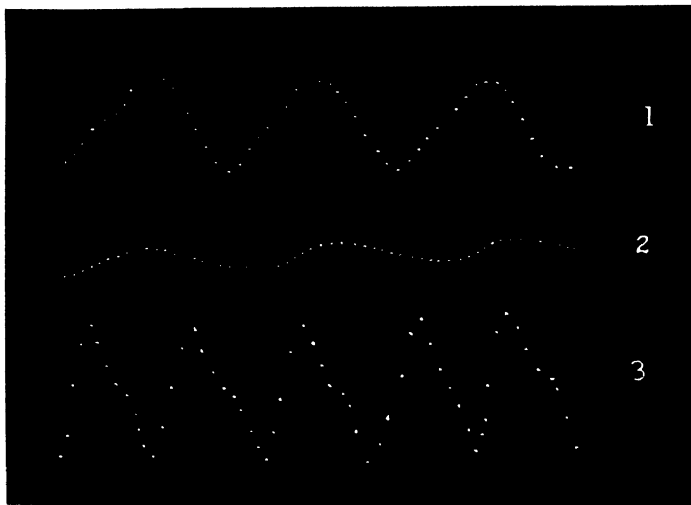


FIG. 8. Effect of variation of temperature on opercular pulsation of a feeble specimen (*Aoria*).

1. Record showing frequency of pulsation at 21.1°C .
2. Depressed pulsation at lower temperature of 12.2°C .
3. Increased amplitude and frequency at 28.3°C .

when the temperature was restored to the original 22.2°C .; and finally when it was raised to 28.3°C .

On account of the vigorous condition of this particular specimen of *Aoria*, the period of its single pulsation at 22.2°C . was as short as 0.45 second, the frequency of respiration being 133 per minute. Lowering of temperature to 12.2°C . caused an arrest of opercular pulsation. On restoration of temperature to the normal 22.2°C . the frequency was restored to the normal value of 133 per minute. Finally, when the temperature was raised to

28.3° C. the frequency of respiration was increased to 171 in place of 133 per minute at 22.2° C., the period of a single opercular pulsation being quickened from 0.45 to 0.35 second. There was but little variation in the amplitude of pulsation (fig. 9).

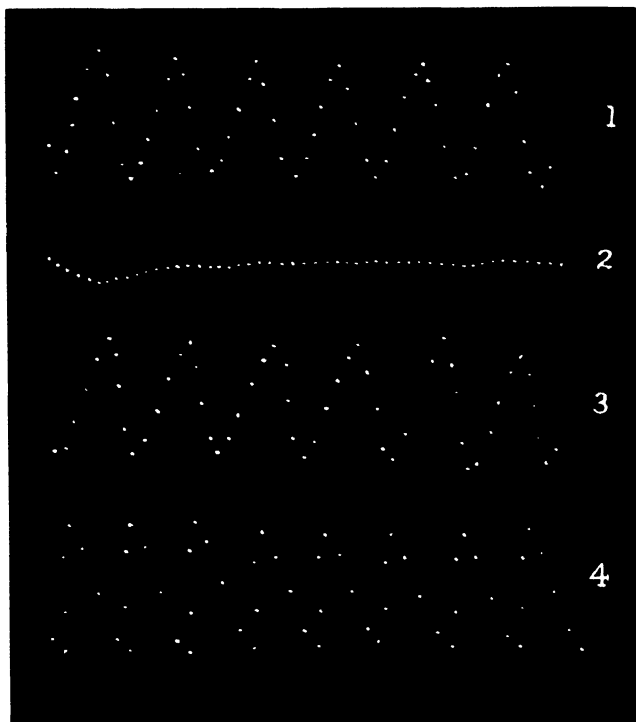


FIG. 9. Effect of cyclic variation of temperature on opercular pulsation of a vigorous specimen (*Aoria*).

1. Normal pulsation at 22.2° C.
2. Arrest of pulsation at 12.2° C.
3. Restoration of the normal rate after return to original temperature 22.2° C.
4. Increased frequency at 28.3° C.

PULSATION OF THE MOUTH

Experiment 28.—The record of the pulsation of the mouth of a specimen of *Aoria* was taken at 22.7° C. The period of a single pulsation, represented by 12 dot-intervals,

was 0.6 second, the frequency being 100 per minute. On lowering the temperature to 12.2°C . there was an immediate diminution of the amplitude and a slowing down of the rate, the time of each pulsation being prolonged to 1.35 second. The most noticeable change in the record is the flattening

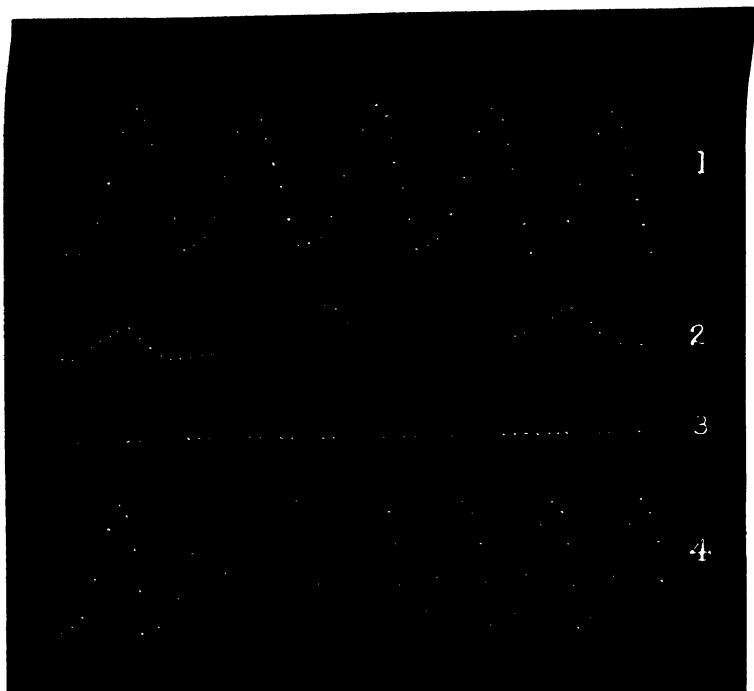


FIG. 10. Effect of variation of temperature on pulsation of the mouth (*Aoria*).

1. Normal pulsations at 22.7°C .
2. Immediate slowing down of rate by lowering of temperature.
3. Arrest at 12.2°C .
4. Increased frequency at 29.4°C .

of the up-curve, which represents the movement of opening of the mouth. Continued exposure to this low temperature caused complete arrest of pulsation. Warm water at 29.4°C . was then substituted. This caused a revival of pulsation, each one of which was completed in the course

of 0.45 second; thus the frequency of pulsation was increased from 100 at 22.7° C. to 133 per minute at 29.4° C. (fig. 10)

The definite results obtained by both the methods of record are that lowering of temperature causes a diminution of the frequency of pulsation or a slowing down of the rate which culminates in an arrest. Rise of temperature, on the other hand, enhances the respiratory activity, as indicated by the increased frequency of pulsation.

2. EFFECT OF ROOT-EXTRACT

The following experiments carried out with *Aoria* relate to action of a feeble dose (1 part in a thousand) and of a stronger dose (1 part in a hundred) of the root-extract of *Millettia*.

OPERCULAR PULSATION

Experiment 29. *Action of a feeble dose.*—The period of a single normal pulsation of *Aoria* was 0.4 second, the frequency being 150 per minute. On application of the dilute extract, the respiratory activity became greatly enfeebled in the course of 15 minutes, as seen in the marked diminution of the amplitude and frequency of pulsation, the frequency being reduced to 67 per minute. The character of the record also underwent a very noticeable change, shown by a long pause between successive pulsations. In each complete pulsation the period of closing was but slightly affected, but the movement of opening of the operculum (down-curve) became very much protracted. Thus while the movement of closing was completed in 0.2 second, it took 0.7 second for the completion of the movement of opening. The pulsations became fully arrested in the course of 30 minutes (fig. 11).

An attempt was next made to revive the respiratory activity by substitution of fresh water. This revival occurred in the course of 5 minutes, as seen in series 4 of fig. 11. The fish was then released and found to be

breathing, though still lying upside-down on its back. Artificial respiration, however, produced a complete revival, after which it swam about vigorously in its normal attitude.

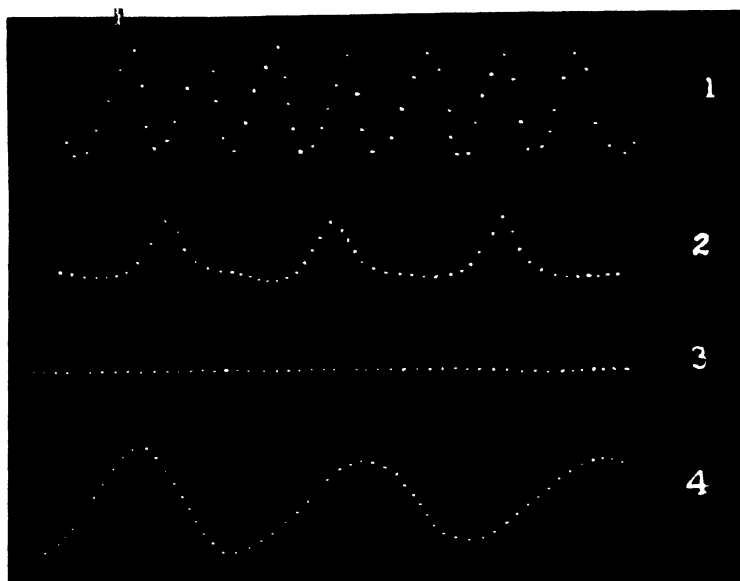


FIG. 11. Effect of dilute root-extract on opercular pulsation (*Aoria*).

1. Normal rate.
2. Depression induced 15 minutes after the application. Note the slowing down of the rate of opening movement of the operculum (down-curve).
3. Arrest of pulsation after 30 minutes.
4. Revival after substitution of fresh water.

Experiment 30. *Effect of a strong dose.*—The changes induced in the rate of respiration now took place with greater rapidity. The normal frequency of respiration of the particular specimen of *Aoria* was 109 per minute. Two minutes after the application of the extract the amplitude of pulsation was reduced, and the frequency lowered from 109 to 100 per minute, and the pulsation came to a stop in the course of 10 minutes after the application (fig. 12). On substitution of fresh water there was a temporary

revival, but in spite of artificial respiration the fish ultimately succumbed.

In order to ascertain whether these observed effects of the root-extract of *Millettia* are of general occurrence, the following experiment was carried out with *Cirrhhina*, the respiratory activity of which is, generally speaking, more sluggish.

Experiment 31. *Effect of dilute extract on Cirrhina.*—



FIG. 12. Effect of strong dose of the extract on opercular pulsation (*Aoria*).

1. Normal rate.
2. Diminished amplitude and frequency of pulsation after 2 minutes.
3. Arrest after 10 minutes.

The dilution employed for this was 1 part in a thousand. The normal frequency of respiration of the fish was 71 in a minute. The immediate effect of the extract was to induce a temporary enhancement of activity in the course of 2 minutes, as shown by the increase of amplitude and of frequency of pulsation, the latter being increased from the original 71 to 92 per minute. But after 10 minutes the amplitude became markedly diminished and the frequency

greatly reduced. After 15 minutes the opercular pulsation became completely arrested (fig. 13).

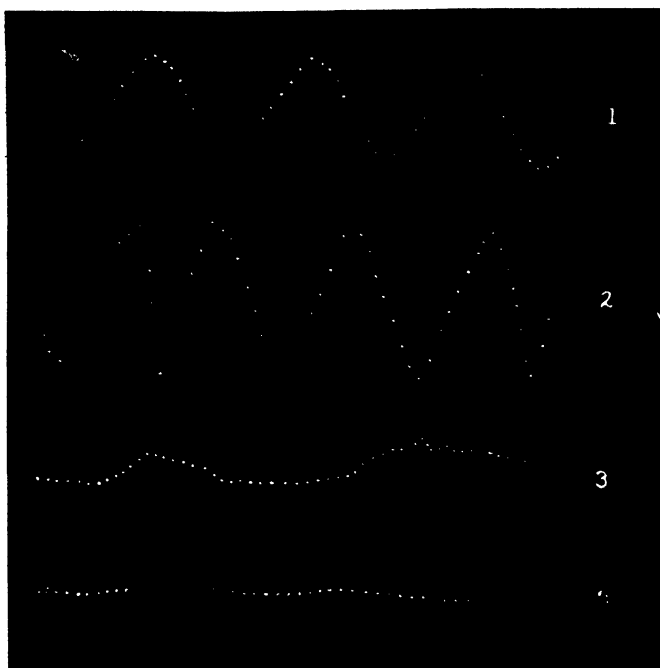


FIG. 13. Effect of dilute root-extract on opercular pulsation of *Cirrhina*.

1. Normal rate.
2. Preliminary stimulation after 2 minutes.
3. Depression after 10 minutes.
4. Arrest of pulsation after 15 minutes.

PULSATION OF THE MOUTH

Experiment 32. *Effect of a dilute extract on opening and closing movements of the mouth.*—The *Aoria* employed in this experiment proved to be extremely susceptible to the action of the extract. The normal period of a single pulsation of the mouth was 0.55 second, the frequency being 109 per minute. On application of a dilute solution of the extract, 1 part in a thousand, a very marked depression occurred in the course of so short a time as 2 minutes.

There was a great diminution of the amplitude of pulsation and a slowing down of the frequency; the period of movement of opening (up-curve) was lengthened from 0.35 to 0.50 second, and the pulsation stopped altogether 5 minutes after the application (fig. 14).



FIG. 14. Effect of dilute extract on pulsation of the mouth (*Aoria*).

1. Normal rate.
2. Effect after 2 minutes. Note the diminution of amplitude and slowing down of the rate of movement of opening (up-curve).
3. Arrest of pulsation in 5 minutes.

3. EFFECT OF ROOT-EXTRACT ON CARDIAC ACTIVITY

It has been stated (*cf.* Experiment 11) that the root-extract causes only a paralysis of the respiratory activity without affecting the pulsatory activity of the heart. A more rigorous demonstration of this fact is afforded by the automatic records of the heart-beat as given by the Resonant Cardiograph.

Experiment 33.—A specimen of *Ophiocephalus striatus* was subjected to the action of the root-extract with the result of complete arrest of its respiratory movements. The fish was then opened and its heart, which was still found to be actively beating, was suitably attached to the Cardio-

graph. The first series of curves in the record show that the rate of the cardiac pulsation was 75 per minute. The next series of the record, taken 2 hours after, also show that the pulsation of the heart had not undergone any decline. The final series of the record were taken after direct application of the extract on the heart itself, and the result shows that this did not induce any noticeable depression (fig. 15). The root-extract which arrests the respiration

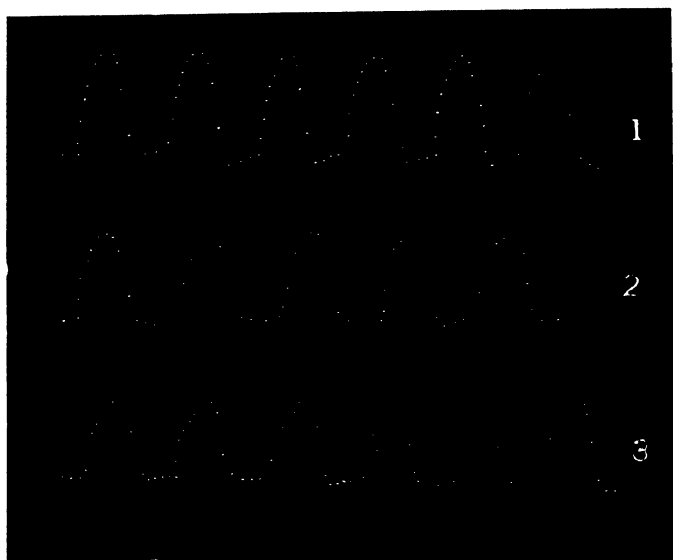


FIG. 15. Effect of the extract on cardiac activity (*Ophioccephalus*).

1. Cardiac pulsation after arrest of respiration by the action of the extract.
2. Persistence of heart-beat after 2 hours.
3. Cardiac activity unaffected by direct application of extract on the heart.

cannot therefore be regarded as a protoplasmic poison, under which all functional activities become abolished.

4. EFFECT OF CARBONIC ACID

The effect of CO_2 is conveniently studied by the application of soda-water charged with an excess of carbonic acid gas. A dilute solution was made by mixing 1 part of such

soda-water with 4 parts of ordinary water; for a strong dose undiluted soda-water was employed.

OPERCULAR PULSATION

Experiment 34. *Effect of dilute CO₂*.—The normal rate of respiration of the specimen of *Aoria* was very uniform, being 150 per minute. The immediate effect of dilute CO₂

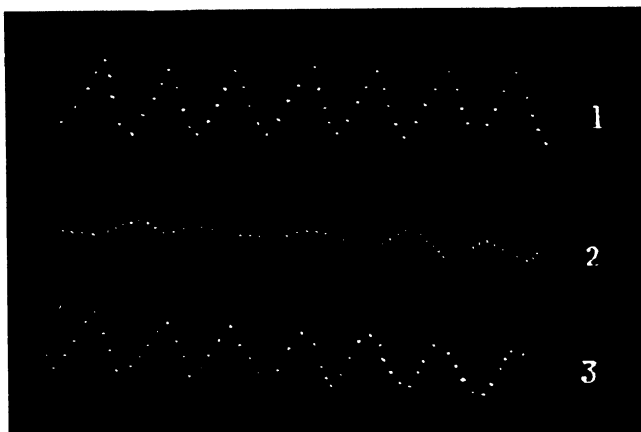


FIG. 16. Effect of dilute Carbonic Acid on opercular pulsation (*Aoria*).

1. Normal rate.
2. Enfeebled pulsation of alternating character after application of dilute CO₂.
3. Revival of pulsations by oxygen.

was often found to be a temporary enhancement of activity and irregularity of a periodic character. But the continued action of dilute CO₂ induced a depression attended by an alternating variation, arrest followed by revival, and this in recurrent series for a considerable length of time; after an interval of about 40 minutes the pulsations became practically arrested. A stream of oxygen was next made to bubble through the water, thus replacing the CO₂. This brought about a complete revival, and the frequency of pulsation was restored to the normal rate of 150 per minute (fig. 16).

Experiment 35. *Effect of strong dose of CO₂.*—The normal frequency of opercular pulsation of the fish *Aoria* was 120 per minute. Application of undiluted soda-water completely arrested the pulsation in as short a time as



FIG. 17. Effect of strong CO₂ on opercular pulsation (*Aoria*).

1. Normal rate.
2. Complete arrest after 3 minutes.

3 minutes (fig. 17). The induced asphyxiation was so profound that the fish could not be revived even after subjecting it to enforced artificial respiration.

PULSATION OF THE MOUTH

Experiment 36. *Effect of dilute CO₂.*—The particular specimen of *Aoria* was somewhat sluggish; a single pulsation was completed in 0.75 second, the frequency being 80 per minute. Two minutes after the application of CO₂ the pulsation became very greatly diminished in amplitude and came nearly to a stop in the course of 5 minutes. Fresh water was then substituted, bringing about a recovery of respiratory activity. The after-effect of dilute CO₂ is seen in the flattening of the top of the curve, which indicates that the fish remained gaping for about 0.2 second before the commencement of the closing movement (fig. 18).

Effect of a strong dose of CO₂.—This induced a pronounced asphyxiation with permanent abolition of respiratory activity.



FIG. 18. Effect of dilute CO₂ on pulsation of the mouth (*Aoria*).

1. Normal rate.
2. Depression of amplitude and slowing down of the rate after 2 minutes.
3. Practical arrest after 5 minutes.
4. Revival after substitution of fresh water. Note flattening of the top of curve as an after-effect.

5. EFFECT OF ETHER

The detailed effects of different anæsthetics will next be described ; in these experiments the solution containing the anæsthetic is substituted for water and the effect observed. Ether may be regarded as a mild anæsthetic, while chloroform in large doses is likely to prove toxic.

OPERCULAR PULSATION

Experiment 37. *Action of dilute Ether.*—The period of a single opercular pulsation of a vigorous specimen of *Aoria* was found to be 0·4 second, the frequency of respiration being 150 per minute. Application of dilute ether for

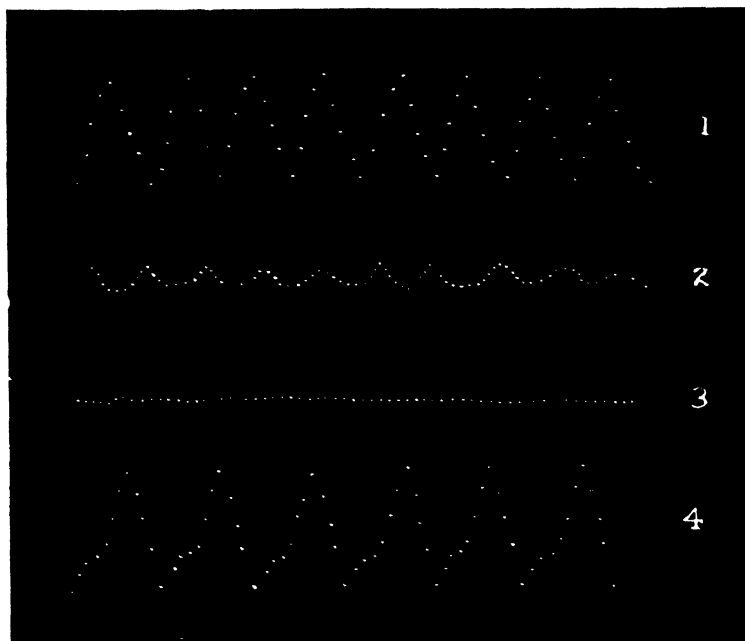


FIG. 19. Effect of dilute Ether on opercular pulsation (*Aoria*).

1. Normal rate.
2. Depression after 12 minutes.
3. Arrest after 18 minutes.
4. Recovery after substitution of fresh water.

12 minutes induced great depression in the amplitude of pulsation, and the respiratory activity became arrested in the course of 18 minutes, after which fresh water was substituted without delay. The fact that the arrest was temporary was proved by the recovery which occurred 7 minutes after the substitution of fresh water. The

amplitude of pulsation after this recovery was sometimes found to be even larger than that at the beginning. A characteristic peculiarity in this particular record is, that the closing movement was not continuous but characterised by a very short intermediate pause (fig. 19).

The fact that only a local paralysis of the respiratory activity had been induced by the anæsthetic has already been demonstrated by a previous experiment, in which the heart of the anæsthetised fish was found to continue its active pulsations (*cf.* Experiment 22).

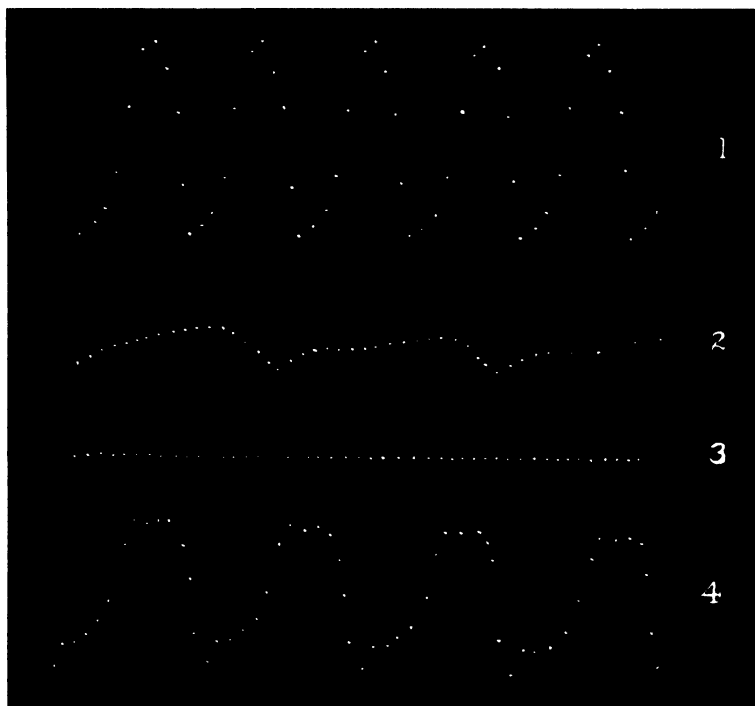


FIG. 20. Effect of dilute Ether on pulsation of the mouth (*Aoria*).

1. Normal rate.
2. Depression and prolongation of the period of opening (up-curve) 2 minutes after application.
3. Arrest of pulsation after 3 minutes.
4. Recovery after substitution of fresh water. Note the after-effect in the flattening of top of curve.

PULSATION OF THE MOUTH

Experiment 38. *Effect of dilute Ether.*—The period of a single pulsation of *Aoria* was 0·5 second, the frequency being 120 per minute. On application of dilute ether the amplitude of pulsation was greatly diminished in the course of 2 minutes, and the period of opening of the mouth was prolonged from the normal 0·3 to 0·7 second. The pulsations became arrested in the further course of a minute. On the immediate substitution of fresh water there was a revival. The characteristic after-effect shown in this record is the flattening of the top of the curve, indicating that the fish remained with its mouth wide open for about 0·15 second before the closing movement was initiated (fig. 20).

6. EFFECT OF CHLOROFORM

Chloroform is but sparingly soluble in water. I first describe the effect of a dilute and then that of a strong solution.

OPERCULAR PULSATION

Experiment 39. *Action of a dilute solution.*—The effect of a dilute solution of chloroform is similar to that of ether, inducing at first a depression and later a stoppage of respiration; the respiratory activity could, however, be restored after proper treatment.

Action of a strong dose.—The effect of this is different from that of a dilute solution. The particular specimen of *Aoria* was but moderately vigorous, the period of a single pulsation being 0·65 second, and the frequency of respiration 92 per minute. After application of the chloroform for 3 minutes, the amplitude of pulsation underwent a great decline, and complete arrest of respiration occurred after 7 minutes (fig. 21). The fish could not, however, be revived from the effect of the strong narcotic.

PULSATION OF THE MOUTH

Experiment 40. *Effect of dilute solution of Chloroform.*—The specimen of *Aoria* employed was very vigorous, the period of a single pulsation was 0.4 second, the frequency being 150 per minute. The solution caused a practical



FIG. 21. Effect of strong Chloroform on opercular pulsation (*Aoria*).

1. Normal rate.
2. Depression induced in 3 minutes.
3. Permanent arrest after 7 minutes.

arrest of pulsation in the course of 3 minutes. On immediate substitution of fresh water the pulsations became revived in the course of 5 minutes. It is interesting to note that the after-effect is very similar to that of dilute ether—that is to say, the top of the curve was flat for a considerable length of time. The fish thus remained gaping for a time, causing delay of the commencement of the closing movement (fig. 22).

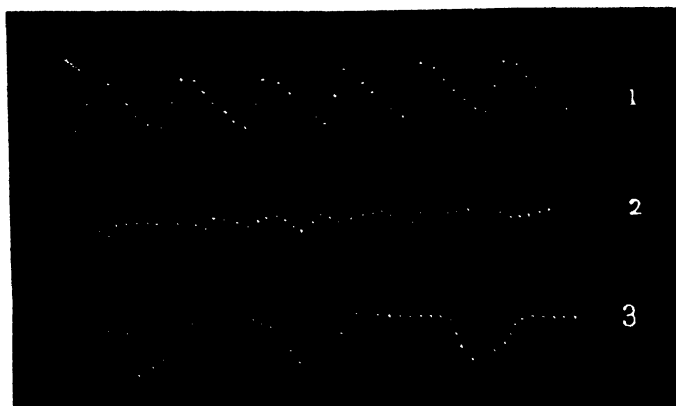


FIG. 22. Effect of dilute Chloroform on pulsation of the mouth (*Aoria*).

1. Normal rate.
2. Marked depression after 3 minutes.
3. Revival on substitution of fresh water. Note flattening of the top of curve as an after-effect.

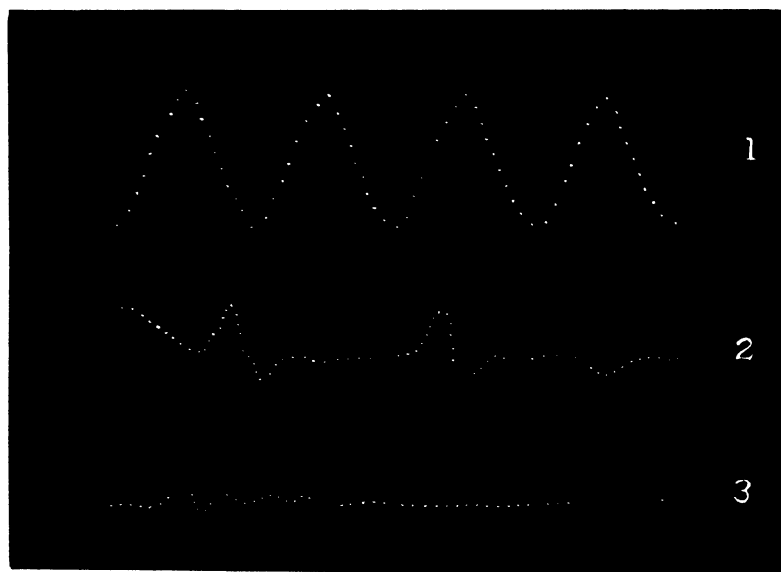


FIG. 23. Effect of Coal Gas on opercular pulsation (*Cirrhina*).

1. Normal rate.
2. Depression after 4 minutes.
3. Permanent arrest of pulsation after 10 minutes.

7. EFFECT OF COAL GAS

The fish is very susceptible to the injurious influence of certain gases. When the bed of a tank or of a stream is full of decaying matter, the gas emitted often causes death of the fish. Similar results follow, when the sludge from a manufactory, containing injurious gases, is discharged into a river. The injurious effect of coal gas is described below.

OPERCULAR PULSATION

Experiment 41. *Effect of Coal Gas.*—After taking the normal record in fresh water, the effect of coal gas was determined. The fish employed for this experiment was *Cirrhina*, the normal single pulsation of which was completed in 0.85 second, the frequency of respiration being 71 per minute. Water charged with coal gas was then substituted for the fresh water; the pulsations became extremely feeble and irregular in the course of 4 minutes and came to a complete stop after 10 minutes (fig. 23). A convulsion of death then passed through the body, after which the fish became rigid.

PULSATION OF THE MOUTH

Experiment 42. *Effect of Coal Gas.*—The period of a single pulsation of the *Aoria* fish was 0.5 second, the frequency being 120 per minute. On application of water charged with coal gas the amplitude became very greatly diminished in the course of 4 minutes and arrested after 8 minutes (fig. 24). As in the previous case there were convulsive movements which preceded death.

8. EFFECT OF SULPHURETTED HYDROGEN¹ GAS

Perhaps there is no other gas which is so poisonous in its action on the fish as H_2S ; this will be understood from the following two experiments.

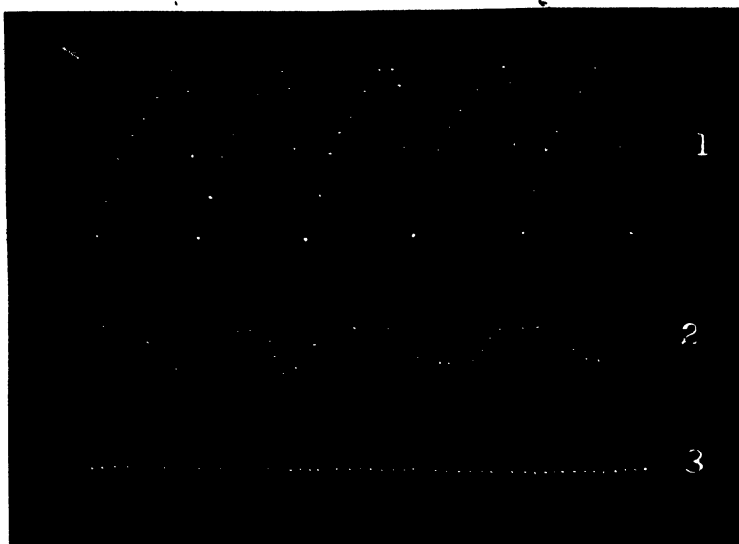


FIG. 24. Effect of Coal Gas on pulsation of the mouth (*Aoria*).
1. Normal rate.
2. Depression after 4 minutes.
3. Permanent arrest of pulsation after 8 minutes.



FIG. 25. Effect of Sulphuretted Hydrogen on opercular pulsation (*Aoria*).
1. Normal rate.
2. Permanent abolition of pulsation in the course of 1 minute.

OPERCULAR PULSATION

Experiment 43. *Effect of H_2S .*—The period of a single pulsation of an *Aoria* fish was 0.55 second, the frequency being 109 per minute. As the bubbles of H_2S were passed through the water, the poisonous effect appeared with extreme rapidity. The pulsations became completely abolished in less than a minute (fig. 25). There was no possibility of revival, the fish being dead.

PULSATION OF THE MOUTH

Experiment 44. *Effect of H_2S .*—The specimen employed for this experiment was even more active than the last.



FIG. 26. Effect of Sulphuretted Hydrogen on pulsation of the mouth (*Aoria*).

1. Normal rate.
2. Rapid abolition of pulsatory activity.

The period of a single pulsation of this specimen of *Aoria* was 0.35 second, the frequency being 171 per minute. The effect of H_2S in abolishing the pulsation of the mouth was as rapid as in the previous case (fig. 26).

9. EFFECT OF CARBON DISULPHIDE

Experiment 45. *Effect of traces of CS₂.*—The solubility in water of this agent is extremely slight, but even traces of it prove to be highly injurious. The experiment was carried out with a specimen of *Aoria*, the period of a single pulsation of which was 0·6 second, the frequency of respiration being 100 per minute. The application of water,

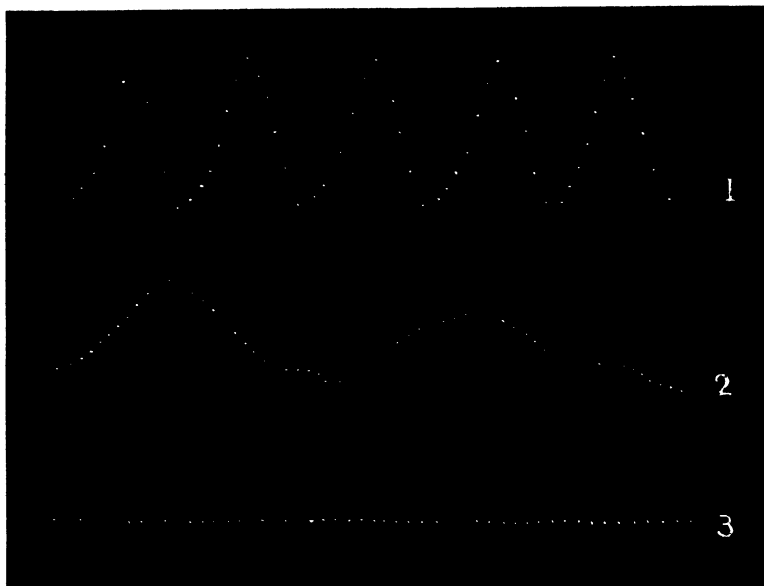


FIG. 27. Effect of Carbon Disulphide on opercular pulsation (*Aoria*).

1. Normal rate.
2. Marked slowing down of the rate in 2 minutes.
3. Permanent arrest after 5 minutes.

containing a minute quantity of carbon disulphide, caused great depression and slowing down of the rate of respiration in the course of 2 minutes. After 5 minutes the opercular pulsation came to a complete stop (fig. 27). The arrest was found to be permanent, as artificial respiration failed to revive the respiratory activity.

PULSATION OF THE MOUTH

Experiment 46. *Effect of traces of CS₂.*—Each pulsation of this specimen of *Aoria* was completed after 0·35 second, the frequency being 171 per minute. On application of the carbon disulphide the respiratory activity became markedly slowed down in the course of 2 minutes and was permanently arrested in 3 minutes (fig. 28).



FIG. 28. Effect of Carbon Disulphide on pulsation of the mouth (*Aoria*).

1. Normal rate.
2. Slowing down of the rate after 2 minutes.
3. Permanent arrest after 3 minutes.

Investigations were next carried out on the action of the well-known depressant *potassium bromide*, and of poisonous agents such as *copper sulphate*, and of *potassium cyanide*.

10. EFFECT OF POTASSIUM BROMIDE

OPERCULAR PULSATION

Experiment 47. *Effect of dilute solution.*—The period of a single pulsation of the fish *Aoria* was 0·45 second, the

frequency being 133 per minute. Application of a 3 per cent. solution of potassium bromide for 4 minutes brought about a progressive diminution of the amplitude of pulsation, the frequency remaining practically unchanged. The pulsations were arrested after 10 minutes (fig. 29). It was, however, found to be possible to revive the respiratory



FIG. 29. Effect of solution of Potassium Bromide on opercular pulsation (*Aoria*).

1. Normal rate.
2. Depression induced after 4 minutes.
3. Arrest of pulsation after 10 minutes.

activity by substitution of fresh water. The solution of potassium bromide thus acts as a depressing and not as a poisonous agent.

PULSATION OF THE MOUTH

Experiment 48. *Effect of dilute solution of potassium bromide.*—The amplitude of normal pulsation of the specimen of *Aoria* was considerable; the period of a single pulsation was 0·5 second, and the frequency 120 per minute.

Application of a 3 per cent. solution of the bromide greatly reduced the amplitude of pulsation in the course of 10 minutes. The top of the curve, representing position of opening of the mouth, is seen to have become flattened. After 15 minutes the pulsations came to a state of standstill (fig. 30). The respiration became revived after substitution of fresh water.



FIG. 30. Effect of solution of Potassium Bromide on pulsation of the mouth (*Aoria*).

1. Normal rate.
2. Depression induced after 10 minutes.
3. Pulsations at standstill after 15 minutes.

II. EFFECT OF SOLUTION OF COPPER SULPHATE

It is a curious fact that a dilute solution of copper sulphate does not produce any immediate poisonous effect. The fish becomes irritated by its action and may sometimes exhibit a preliminary stimulation. But long-continued action brings about depression and death. Very dilute solutions, which destroy algal growth, appear to have no effect on the fish.

OPERCULAR PULSATION

Experiment 49. *Action of moderately dilute CuSO_4 solution.*—The period for a single pulsation of *Aoria* was 0.5 second, the frequency being 120 per minute. After

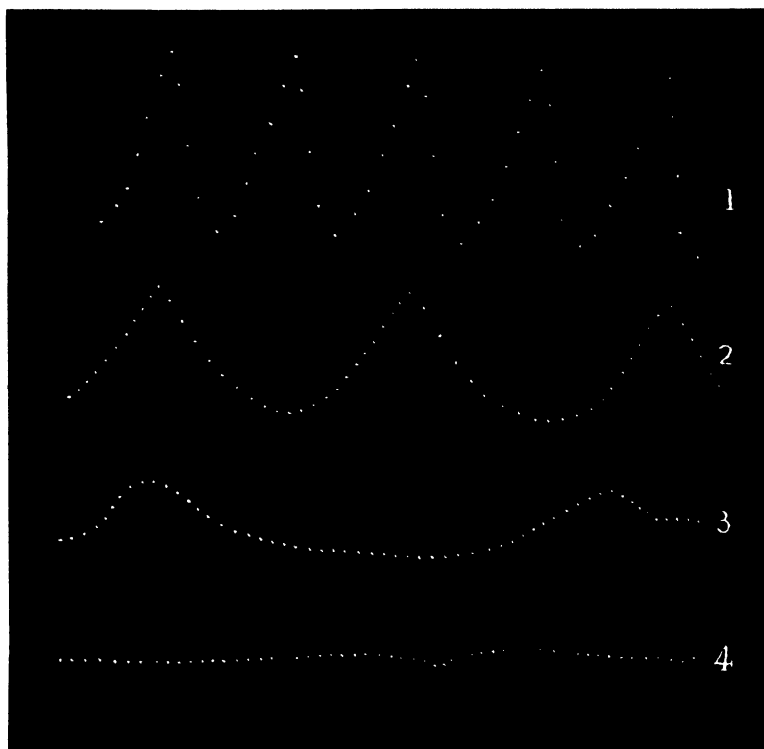


FIG. 31. Effect of solution of Copper Sulphate on opercular pulsation (*Aoria*).

1. Normal rate.
2. Depression of the rate after 15 minutes.
3. Greater depression after 25 minutes.
4. Permanent arrest of pulsation after 30 minutes.

application of 1 per cent. solution of copper sulphate the pulsations underwent a slow decline. The rate became depressed to about three-fifths in 15 minutes, and to two-fifths in 25 minutes. The movement of opening also became

greatly prolonged. After 30 minutes there was an arrest of respiratory activity (fig. 31), which was found to be permanent, since it was impossible to revive it.

PULSATION OF THE MOUTH

Experiment 50. *Effect of moderately dilute solution of CuSO_4 .*—The specimen of *Aoria* was very active. The

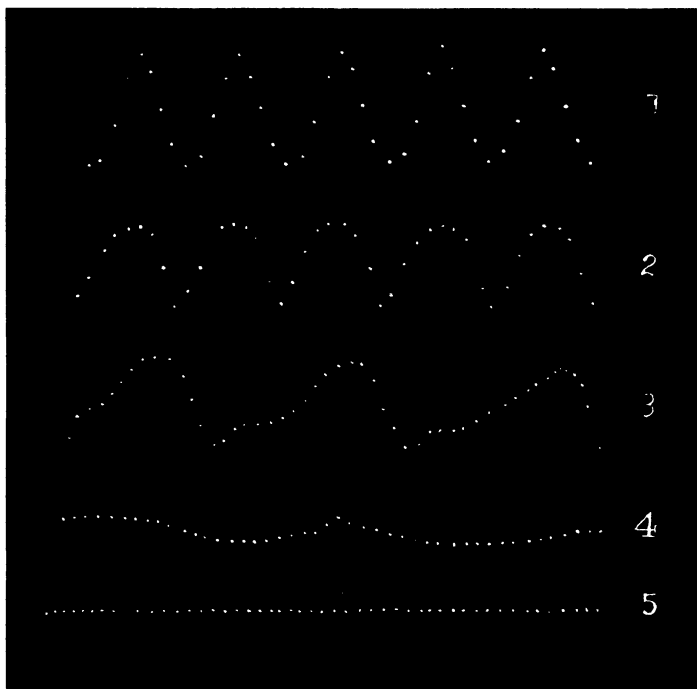


FIG. 32. Effect of CuSO_4 on pulsation of the mouth (*Aoria*).

1. Normal rate.
2. Diminution of amplitude of pulsation after 3 minutes.
3. Prolongation of movement of opening after 15 minutes.
4. Diminution of amplitude and frequency after 20 minutes.
5. Permanent stoppage after 30 minutes.

period of a single pulsation was 0.4 second, the frequency being 150 per minute. On application of 1 per cent. solution of the copper sulphate the amplitude became

reduced in the course of 3 minutes, the top of the curve exhibiting a flattening. After 15 minutes the frequency was reduced to about three-fifths, and the time taken to complete the movement of opening was prolonged from 0.2 to 0.6 second. After 20 minutes both the frequency and the amplitude exhibited a decline, and the pulsations came to a permanent stop after 30 minutes (fig. 32).

12. EFFECT OF POTASSIUM CYANIDE

OPERCULAR PULSATION

Experiment 51. *Action of KCN Solution.*—This is found to be extremely toxic in its action. The normal



FIG. 33. Effect of dilute solution of Potassium Cyanide on opercular pulsation (*Aoria*).

1. Normal rate.
2. Marked depression of amplitude and frequency after 2 minutes.
3. Permanent arrest after 5 minutes.

period of a single pulsation of *Aoria* was 0.7 second, the frequency of respiration being 86 per minute. Under such a dilute dose as 1 part in a thousand, the pulsations became

greatly enfeebled in the course of 2 minutes, and arrested after 5 minutes, from which there was no possibility of revival (fig. 33). Not only was the respiratory activity permanently abolished, but the internally diffused poison also abolished the cardiac pulsation.

PULSATION OF THE MOUTH

Experiment 52. *Effect of dilute solution of KCN.*—Each pulsation of the *Aoria* fish was completed in 0.65 second, the

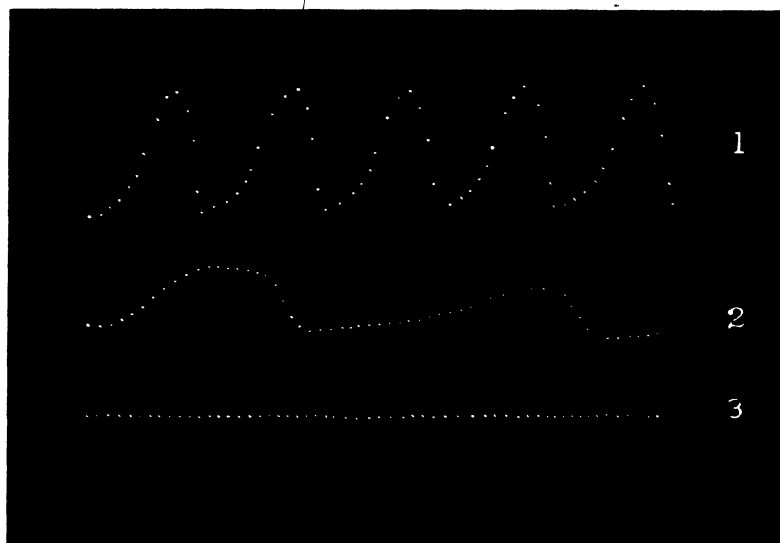


FIG. 34. Effect of dilute solution of Potassium Cyanide on pulsation of the mouth (*Aoria*).

1. Normal rate.
2. Depression after 1 minute.
3. Permanent abolition after 3 minutes.

frequency being 92 per minute. Application of the dilute solution of the cyanide caused an immediate depression, the period of the opening movement (up-curve) becoming greatly prolonged. A permanent abolition of the respiratory activity occurred in the course of 3 minutes (fig. 34).

How essentially different is the effect of the root-extract to that of the protoplasmic poison will be clearly demonstrated in the two following series of investigations on the contrasted effects induced by their injection into the fish.

13. EFFECT OF INJECTION OF ROOT-EXTRACT SOLUTION

Before describing the effect of injection of the root-extract, I will briefly refer once more to its effect on the respiration of the fish. A short duration of application of the solution has already been shown to arrest the respiratory activity, which can be revived on substitution of fresh water. A prolonged application of the extract, however, induces a permanent arrest of respiration, resulting in the death of the fish. The two experiments which follow were carried out with equally vigorous specimens of *Aoria*: (a) on the effect of prolonged external application of the dilute extract; and (b) on the effect of injection of the extract.

Experiment 53. *Effects of external application and of injection of root-extract:*

(a) *Effect of prolonged external application of extract.*—The normal respiratory activity of the fish in water was 108 per minute. It was next placed in a vessel containing dilute solution of the extract, one part in a thousand, and the results, given in the following table, are essentially similar to those obtained previously (*cf.* Table II).

TABLE VII.—EFFECT OF ROOT-EXTRACT ON RESPIRATION.

Normal respiratory activity	.	.	.	180 per minute
7 minutes after application	.	.	.	120 " "
15 " " "	.	.	.	90 " "
45 " " "	.	.	.	Arrest

There was a partial upset in the course of 15 minutes, the paralysis being complete after 20 minutes.

(b) *Effect of injection of the root-extract.*—In this experiment 1 c.c. of 1 per cent. solution of the root-extract was injected into one flank of a specimen which was in every way similar to that of experiment (a). After injection, the fish was released into a vessel of fresh water. It was a very striking fact that the injection of the root-extract had no effect on the normal physiological activity of the fish, which, kept under observation for several days, continued to swim about with normal vigour.

From these results it is clear that the injection of the root-extract has practically no effect in modifying the general physiological activity of the fish. When the extract acts upon the gills it only causes a local paralysis of the respiratory organ.

14. EFFECT OF INJECTION OF PROTOPLASMIC POISON

The contrasted effect of injection of protoplasmic poison will now be described. *Potassium Cyanide* has already been shown to act as a general protoplasmic poison.

Experiment 54. *Effect of injection of KCN solution.*—The experiment was carried out on the same day as the previous experiments, with three equally vigorous specimens. In a typical case the respiratory activity was 120 per minute. After injection of dilute solution of KCN on one of its flanks, the fish was released in a vessel of fresh water. Its vital activity was now observed to undergo a rapid decline, and the fish became upset, floating on its back upside-down. A violent spasm then occurred, indicative of death.

The injected poisonous solution soon became diffused, and abolished the physiological activities of all its organs. This was rendered evident by the post-mortem examination of the fish, which showed that the muscles had become stiff and rigid, that the gills were congested with blood, and that the heart had stopped at diastole.

SUMMARY

Large quantities of fish used to be captured in the hill-streams of Darjeeling by the Lepcha fishermen, the fish

being supposed to be poisoned by application to the stream of extracts from stems, fruits, and roots of various plants. Among the most effective of these is the root of *Millettia pachycarpa*.

Under the action of plant-extracts, all varieties of fish became paralysed and moribund ; lying passively on their backs upside-down, they were carried by the stream and easily captured.

The fish, though regarded as poisoned, prove, nevertheless, to be safe as human food. Clearly the plant-extract works not as a diffuse protoplasmic poison, but acts locally on some definite organ and function. The present investigation was undertaken to ascertain what this organ might be, and how it is rendered inactive.

The marked external symptoms that appear in sequence under the action of the root-extract are (1) the partial loss of balance, on account of which the fish lies on its side ineffectively trying to right itself, and (2) the complete loss of balance, when the fish lies upside-down in a condition of passivity. The first of these is described as the condition of partial, and the second as that of complete paralysis. The minimally effective dose of the extract is a solution of 1 part in a thousand (Experiments 3, 6, 12).

These symptoms do not afford quantitative means for the determination of the physiological reactions induced by the extract. It is shown that the physiological depression can be more accurately gauged by observation of the concomitant changes in the respiratory activity of the fish, as effected by the periodic opening and closing movements of its opercula and mouth.

In the Method of Eye-Observation the effect of the extract is found from the variation induced in the normal frequency of opercular pulsations of the fish. The frequency remains uniform under normal conditions for a considerable length of time, even when the fish is kept immersed under water. The change induced in the rate of respiration, under the action of the extract, is observed at frequent intervals.

This method of discontinuous eye-observation does not,

however, afford full knowledge of the respiratory mechanism, and of the characteristic changes induced in it under the action of external agencies. For this two different methods of Automatic Record of the Respiratory Movements have been perfected: (1) that of Opercular Pulsations, and (2) that of Pulsations of the Mouth. The respiratory curve is obtained by the Resonant Respirograph, in which the record consists of a succession of dots inscribed at intervals of 0.05 second. The error of friction is completely removed, and the time-relations of the two phases in each curve, representing the movements of opening and of closing, is obtained with unprecedented accuracy from the dotted record itself.

The effect of physical variation of temperature is, that lowering of temperature causes a diminution of frequency or a slowing down of the rate of pulsation, culminating in an arrest. This occurs at or about 12° C. In Bengal this is the minimum temperature in winter, at which the fish *Aoria* and several others enter into a state of hybernation. In contrast with the effect of fall of temperature is that of thermal rise, which enhances the rate of respiration (Experiments 26, 27, 28).

In regard to the effect of the solution of root-extract on the respiratory mechanism, the records show a continuous diminution of both the amplitude and frequency of pulsation; and further a long pause intervenes between successive pulsations, in each of which the period of closure is but slightly affected, while that of opening is very much protracted (Experiments 29, 30, 31, 32).

The following facts prove that the extract cannot be regarded as a general protoplasmic poison, since—

- i. The fish can be revived by artificial respiration.
- ii. The captured fish can safely be eaten, not only by different animals, but also by human beings.
- iii. The extract, when introduced into the stomach of the fish or of the guinea-pig, produces no evil effect (Experiments 9, 10).
- iv. While a protoplasmic poison by internal diffusion causes abolition of the activity of various organs,

the root-extract has no such effect ; it merely causes a local paralysis of the respiratory activity. In a specimen of *Ophiocephalus* that had been rendered inert by the action of the extract, the cardiac pulsations remained persistent for several hours. Moreover, direct application of the extract on the heart had no effect on its normal activity (Experiment 33).

The different gases and solutions which have a fatal effect on the fish are :

Coal Gas.—Water charged with coal gas causes a great enfeeblement of the opercular pulsation and of the pulsation of the mouth, the respiration being permanently arrested in the course of 10 minutes. A convulsive movement of the whole body occurs, preceding the rigidity of death.

Sulphuretted Hydrogen.—This is extremely poisonous in its action. The respiratory activity, as indicated both by the opercular pulsation and the pulsation of the mouth, becomes completely abolished in the course of time as short as a minute (Experiments 43, 44).

Carbon Disulphide.—The records of opercular pulsation and that of the mouth show that the respiratory activity is abolished by a mere trace of this agent (Experiments 45, 46).

Copper Sulphate Solution.—Very dilute solutions of this substance, which destroys algal growth in the water, does not produce any fatal effect on the fish. Continued application of a 1 per cent. solution, however, causes a slow decline of respiratory activity. This is clearly shown in the automatic records of pulsations of the operculum and of the mouth. The amplitude of pulsation undergoes a decline, and the period of movement of opening becomes greatly protracted. The respiration becomes finally abolished in the course of 30 minutes (Experiments 49, 50).

Potassium Cyanide.—External application of this protoplasmic poison proves to be toxic, even in a dilution of 1 part in a thousand. The pulsations of the operculum and those of the mouth exhibit an immediate depression in the course of 2 minutes; the respiratory activity becomes abolished after 5 minutes. In contrast with the localised action of the root-extract on respiration, the effect of this poison is widespread; for after becoming internally diffused it also causes an abolition of the cardiac activity (Experiments 51, 52).

Effect of Injection.—The contrasted effects of the root-extract and of protoplasmic poison like KCN are strikingly demonstrated by the results which follow the injection of the two solutions into the fish. The injection of the root-extract has no harmful effect on the fish, whereas the internal diffusion of the KCN solution abolishes the vital functions of all its organs (Experiments 53, 54).

Asphyxiation under different agents.—A mild narcotic like ether, in a moderate dose, induces a local paralysis of the respiratory activity. The pulsations of the opercula and of the mouth become arrested in the course of a few minutes. Substitution of fresh water, however, revives the activity. The fact that a moderate dose of ether induced an inactivation only of the respiratory organ of the fish was proved by the persistence of the cardiac activity. In all these respects the action of this mild anæsthetic is similar to that of the root-extract (Experiments 21, 22, 37, 38).

The above facts justify the inference that the root-extract induces inactivation of the respiratory mechanism which causes asphyxiation, and this asphyxiation ultimately brings about the death of the fish. This conclusion is further verified by the effects of different means of asphyxiation such as :

- i. *The asphyxiation produced by enforced closure of the mouth and opercula*.—The outward symptoms induced were the same as those produced by the

extract. The fish lost its balance and lay on its side ; later the upset was more complete, when it lay upside-down. It revived after removal of the obstruction to free breathing, as also after artificial respiration (Experiment 18).

- ii. *The asphyxiation produced by water charged with Carbonic Acid gas.*—A moderate quantity of CO_2 in the water causes gradual asphyxiation, as indicated by the depression of respiratory activity. The pulsations of the opercula and of the mouth become enfeebled and finally arrested. There is, however, a revival of respiratory activity when fresh water is substituted without delay, or when a stream of oxygen is made to replace the carbonic acid in the water. Excess of CO_2 , on the other hand, produces so intense an asphyxiation that the fish cannot be revived (Experiments 34, 35, 36).

A local paralysis which involves the inactivation of the respiratory mechanism is effected by the action of the root-extract in an essentially similar manner. The proximate cause of death under the action of the plant-extract is therefore asphyxiation.

III.—THE MOTOR PARALYSIS OF FISH INDUCED BY LOCAL APPLICATION OF SALT

BY

J. P. SIRCAR, B.Sc., M.B., CH.B., AND N. N. DAS, M.B., M.Sc.

THE scaleless fish *Clarias batrachus* is highly valued as nutritious food, specially for invalids. Brought to the market from a distance in small vessels, it can remain alive for a considerable length of time, even without access to water. The fish is provided with hard and sharp-pointed pectoral fins which are used for defensive purposes. When handled without special precautions, it strikes with the fins, thus inflicting very painful, if not dangerous and festering wounds.

The fisherwomen state that the fish can be rendered harmless by the application of ordinary salt, NaCl, on its neck. It then becomes so paralysed that, even when roughly handled, it loses its power to strike back. This particular effect will, for convenience, be described as salt-paralysis, which results in the temporary abolition of the moto-excitability of the fish.

EFFECT OF APPLICATION OF SALT ON THE NECK

The fish taken out of the water, and placed on the ground, wriggles and moves about by muscular action in which its body and fins participate (fig. 35). After application of the salt its muscular movements become arrested. That this is due to the paralysis of moto-excitability can be verified by the application of various testing stimuli.

Experiment 55. *Transmission of the paralysing effect.*—It is a striking fact that the paralysis, induced by the salt

applied at the neck, does not remain localised at the point of application, but travels to a distance, point after point, at a moderately slow speed. The body of the fish, including

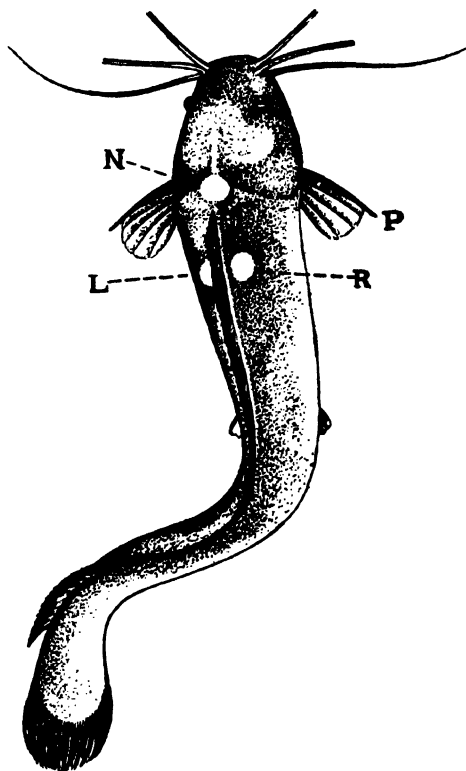


FIG. 35. Mode of movement of *Clarias batrachus*.
 P, hard and sharp-pointed fin for striking. The points of application of salt—N on the neck, R and L on the right and left flanks—have been left white in the figure.

its flanks, becomes inert in the course of 5 minutes, and the fish is rendered incapable of propelling itself forward by any wriggling movement, nor can it strike back when subjected to rough handling.

Experiment 56. *Inability to right itself*.—Under normal conditions the fish actively opposes any attempt to place it

upside-down; when thus inverted it immediately turns over by its muscular action, bringing about restoration to its normal position. But after the application of the salt on the neck, the fish becomes so inert that it remains completely passive in any position in which it may have been placed.

Experiment 57. *Insensitiveness to a prick.*—When the fish, after the application of salt, is struck with a sharp needle on one or the other flank, it exhibits no active movement of response.

Experiment 58. *Insensitiveness to chemical irritants.*—The fish is normally very sensitive to the action of a chemical irritant. Thus when the tip of a pointed glass rod, moistened with sulphuric acid, is brought time after time into contact with any part of the body, the fish gives a violent twitch, which is a reliable test of its normal irritability. But after the application of the salt at the neck, the power of response by mechanical movement becomes completely arrested.

EFFECT OF APPLICATION OF SALT ON ONE FLANK OF THE FISH

The transmission of the induced paralysis is still more strikingly demonstrated by the application of salt, not on the neck but on the right or the left flank of the fish. When the salt is applied on the neck, the transmission is symmetrical on both sides, the paralysis occurring on both the flanks of the fish at about the same time. But the reaction becomes asymmetrical when the salt is applied to one flank only, say to the right. In such a case the whole length of the proximal right side is likely to be the first to become paralysed, since the paralysing action would be expected to take a longer time to cross over to the opposite or distal side. This inference is verified as follows:

Experiment 59. *Period of transmission from the right to the left flank.*—The salt was at first applied to the point at the right flank. The testing stimulus employed was a chemical irritant, a drop of sulphuric acid on the tip of a

glass rod. The time required for loss of sensibility of the entire length of the proximal right side of the fish was found to be 4 minutes, while the left side still remained sensitive. It was not till 8 minutes had elapsed after the application of the salt to the right, that the paralysis also spread to the left side. Hence the period of transmission of the benumbing action from the proximal across to the distal side was 8 - 4 or 4 minutes.

Experiment 60. *Transmission from the left to the right flank.*—The point of application of the salt was transferred from the right to the left flank of a different specimen. Here also the proximal left side became insensitive earlier, being paralysed in the course of 3 minutes. The insensibility of the distal side occurred 6 minutes after the application, the period of transmission across the fish being in this case 3 minutes.

It would thus appear that the paralysing effect creeps slowly from point to point, and that the transmission is relatively quicker in the longitudinal than in the transverse direction, as shown by the slower passage of the effect from one to the opposite flank of the fish.

The question next arises whether the paralysing effect of salt is confined to the *Clarias*, or whether it is of general occurrence. With the idea that other fishes without scales might probably exhibit the same characteristic effect, the following experiments were carried out with *Saccobranchus fossilis* and *Aoria tengara*.

Experiment 61. *Effect of salt on Saccobranchus fossilis.*—This fish is more or less of the same size as *Clarias*, i.e. 15 cm. in length. Application of the salt to the neck caused paralysis of both sides in the course of 7 minutes. Unilateral application of the salt on one flank caused an earlier paralysis of that side in about 5 minutes. Additional time was required for transverse transmission of the effect from one side to the other, the period of which varied in different specimens from 5 to 6 minutes.

Experiment 62. *Effect of salt on Aoria.*—The length of a middle-sized fish is about 8 cm. ; being thus relatively short, the application of the salt at the neck induced paralysis on

both sides in the shorter period of 3 minutes. The effect of application of salt at one side was, however, transmitted to the opposite side at a comparatively slow rate, the period of transmission being 6 minutes. The following tabular statement gives the average result of a large number of experiments carried out with the three different species of fish.

TABLE VIII.—GIVING PERIOD OF TRANSVERSE TRANSMISSION OF SALT-PARALYSIS.

Specimen	Time for paralysis of proximal side	Time for paralysis of distal side	Time for transverse transmission
<i>Clarias</i> . .	3.5 minutes	7.0 minutes	3.5 minutes
<i>Saccobranchus</i> . .	5.0 „	10.5 „	5.5 „
<i>Aoria</i> . .	3.0 „	9.0 „	6.0 „

TEMPORARY CHARACTER OF SALT-PARALYSIS

Experiment 63. *After-effect of removal of salt.*—It has been shown that paralysis induced by the action of salt, applied on the neck or on either flank, was so great that the fish was incapable of exhibiting any responsive movement under various modes of mechanical and chemical stimulation. Its power of respiration, however, remained unimpaired. The salt was then washed off and the fish replaced in water, when its moto-excitability became fully restored in the course of a few hours. The fish now swam about with its normal vigour, being none the worse for the paralysis that had been artificially induced. A second application of the salt induced the same series of results as at the beginning.

Effect of electric stimulation.—The salt-paralysis has been shown to bring about arrest of the power of movement in response to mechanical and chemical stimulation. The following investigations were undertaken to ascertain the effect of electric stimulation on a fish after application of the salt.

Experiment 64. *Effect of a constant current.*—A voltaic battery, giving 10 volts, is employed for the constant current. The fish is suspended by a metallic clip, which constitutes the upper electrode; the lower electrode is a pin which is thrust into the tail of the fish. By pressing a key a constant current is sent through the fish; a responsive movement of the tail then occurs every time the current is made.

Experiment 65. *Effect of an induction current.*—Induction shocks from a secondary coil were also passed through the fish by means of the two electrodes. These shocks also caused a twitching movement.

It would thus appear that the paralysing effect of salt, though highly effective in regard to mechanical and chemical stimulation, is not equally effective in regard to electric stimulation.

SALT-PARALYSIS OF FISH WITH SCALES

Since the arrest of moto-excitability is apparently brought about by the local absorption of the salt by the skin and by the slow transmission of the paralysing effect to a distance, it would seem probable that a fish having scales would but feebly exhibit this characteristic effect; for the interposed scales would obstruct the absorption of the salt by the skin and therefore delay the reaction. This inference is fully justified by the following experiments.

Experiment 66. *Effect of salt on a scaly fish.*—The experiments were carried out with *Anabas testudineus* and *Ophiocephalus striatus*, which are taken as representatives of fish with scales. Application of salt on these fish did not induce the paralysing effect until after a very long period. In order to facilitate the absorption of the salt by the skin, the scales were removed from small areas of the neck and of the two flanks. After the local application of the salt at these small areas the fish became perfectly inert throughout in the course of 7 to 10 minutes.

The results that have been described prove that the induced salt-paralysis is of general occurrence; since the

various fishes enumerated, with or without scales, exhibit similar reactions.

CARDIAC ACTIVITY UNAFFECTED BY EXTERNAL APPLICATION OF SALT

It has been explained that the application of salt on the skin induces only a paralysis of the moto-excitability of the fish, and that it causes no derangement of its respiratory

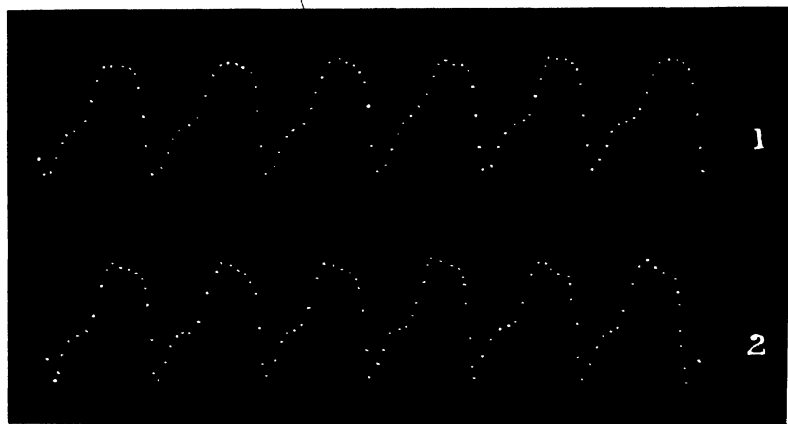


FIG. 36. Records of cardiac pulsation of *Ophiocephalus* rendered inert by external application of salt.

1. Pulsations recorded at the commencement.
2. Pulsations remained constant after 2 hours.

process. It will now be shown that the external application of salt has no effect on the internal activities of the fish, such as the normal pulsations of the heart. The study of the cardiac activity of the fish thus becomes possible under ideal conditions which closely approximate the normal. The fish, after application of the salt on the neck and on the flanks (after removal of the scales when necessary), is laid on its back, in which position it remains quiescent. A small incision is then made for connecting, by means of a thin thread, the apex of the heart with the short arm of the Resonant Recorder. The successive dots in the record are at the usual intervals of 0.05 second.

Experiment 67. *Record of normal cardiac activity.*—The following experiment was carried out with *Ophiocephalus*, which is very hardy and can live for days outside water. Two series of records of cardiac pulsation of the fish, the second taken after an interval of two hours, are reproduced in fig. 36. The important feature of these pulse-records is their remarkable uniformity, which is maintained

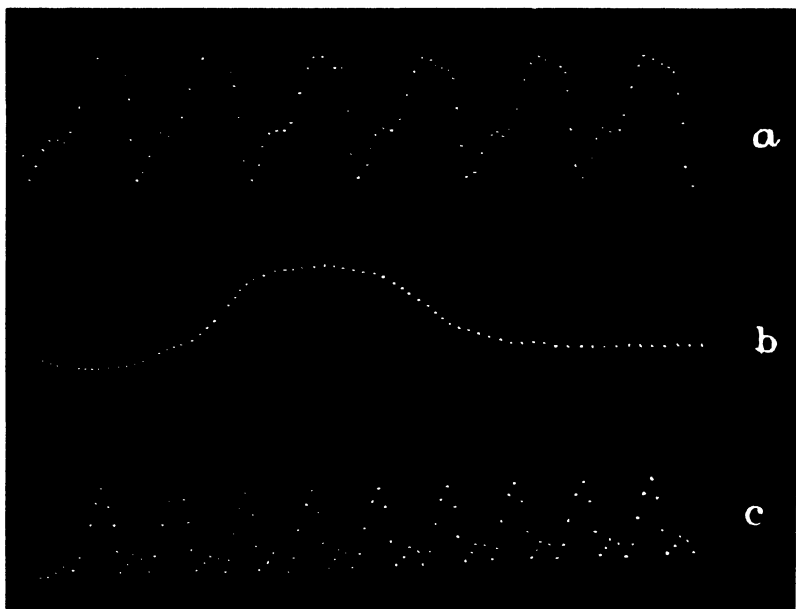


FIG. 37. Effect of variation of temperature on cardiac activity of *Ophiocephalus*.

- a. Record at temperature of 24°C .
- b. Depression at temperature of 6.6°C .
- c. Enhanced activity at 38°C .

constant for several hours. Inspection of the first series of records shows the following characteristics: (a) the ventricular pulse is preceded by the auricular; (b) the top of the curve exhibits a systolic plateau representing a duration of 0.2 second. The period of a single pulsation is 0.8 second, the frequency being 75 per minute. The second series, taken, as already stated, after 2 hours, is

practically the same as the first in every particular. This constancy proves that the conditions of the experiment are nearly as normal as in the intact fish.

The pulse-records are not only characterised by the species of the fish, but are also dependent on the tonic condition of the individual specimen, and subject to modification under change of environmental conditions.

Experiment 68. *Effect of variation of temperature on cardiac activity.*—By the method above described, great facilities are offered for the investigation of the physiological change induced by external agencies. An illustration of this is the following experiment on the effect induced by variation of temperature, whether a thermal fall or a thermal rise. The first series of records was taken with *Ophiocephalus* when the temperature was 24° C., at which a single pulsation was completed in 0.85 second, the frequency being 70.5 per minute. On lowering the temperature to 6.6° C. the pulsatory activity underwent a marked diminution; the post-diastolic pause was 5.1 seconds, and the frequency slowed down to about 12 per minute. The temperature was next raised to 38° C.; the sluggishness which attended the fall of temperature was now removed, and the frequency of pulsation became enhanced to 100 per minute in the place of 70.5 at 24° C. (fig. 37).

EFFECT OF SALT ON CARDIAC PULSATION

The moto-excitability to external stimulation has been shown to become arrested after the application of salt, the excitability being restored after its removal. The question next arises whether automatic cardiac pulsation is also affected by the local application of strong solution of salt on the heart.

Experiment 69. *Effect of direct application of salt on the heart of Ophiocephalus.*—The normal pulsation of this particular specimen was first recorded and found to be very uniform. The usual characteristics, the auricular pulse and the systolic plateau, were also present here as in the previous case. The period of a single pulsation was

0.9 second, the frequency being 67 per minute. After the application of strong solution of salt on the heart, the amplitude of pulsation exhibited a continuous diminution till a practical arrest occurred in the course of 5 minutes, as seen in the second series of records. If the salt is washed off without the least delay, there was a revival which, as in

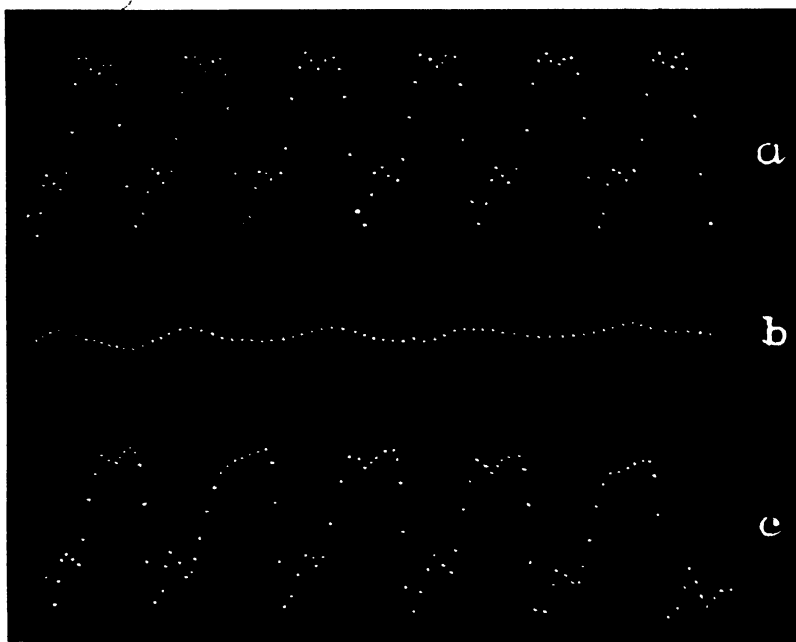


FIG. 38. Effect of direct application of salt on the heart of *Ophiocephalus*.

- a. Normal record.
- b. Practical arrest in 5 minutes after application.
- c. Revival of activity after quick removal of salt.

the present case, was fairly complete 15 minutes after the removal of the salt. The amplitude of pulsation became almost as large as the normal at the beginning, though the frequency was still a little slow, being 57 per minute (fig. 38).

In order to show that the effect of salt on the heart of other fishes is essentially the same, the following experiment was carried out with *Anabas testudineus*.

Experiment 70. *Effect of salt on the cardiac pulsation of Anabas.*—The pulsation of the heart in this fish is a little quicker than in *Ophiocephalus*; in other respects there is considerable similarity between the two. The first series of normal records in fig. 39 shows that the auricular is followed by the ventricular pulsation. The apex of this particular curve is pointed and not flat. The period of each single

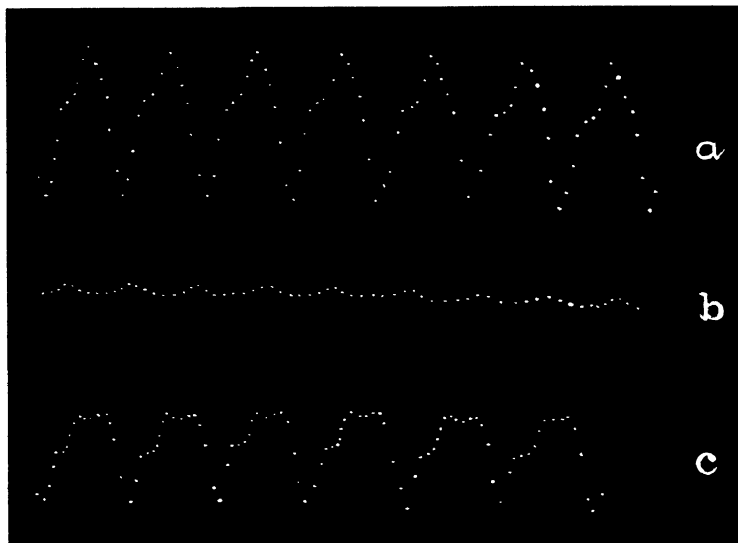


FIG. 39. Effect of direct application of salt on the heart of *Anabas*.

- a. Normal pulsation.
- b. Practical arrest after 3 minutes' application of salt.
- c. Revival of pulsation after quick removal of salt.

pulsation is 0·7 second, the frequency being 85 per minute. After application of salt on the heart there was a practical arrest in the course of 3 minutes, as seen in the second series of records. After the removal of the salt there was a recovery in the course of 15 minutes. The after-effect was a slight diminution of the amplitude; the frequency of the pulsation became almost the same as at the beginning. The top of the curve exhibited a slight change, which instead of being pointed showed a systolic plateau.

The next object of inquiry is whether the above effect is true only of the cardiac pulsation of fish, or whether parallel effects can be observed in the pulsation of the heart of other animals, such as that of the frog.

Experiment 71. *Effect of salt on pulsation of frog's heart.*—The normal record is given in the first series of fig. 40. The period of a single pulsation was 0·6 second, and the frequency 100 per minute. The application of

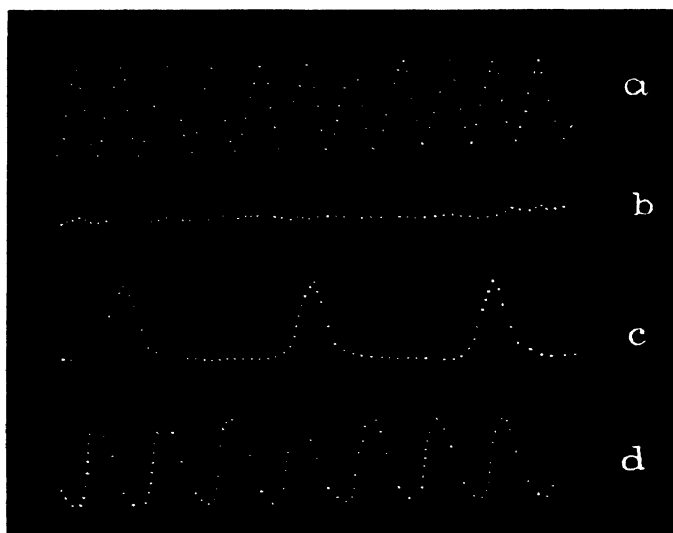


FIG. 40. Effect of direct application of salt on Frog's heart.

- a. Normal pulsation.
- b. Arrest after application of salt.
- c. Slow revival in the course of 5 minutes after removal of salt.
- d. More complete revival after 15 minutes.

strong solution of salt on the heart caused an arrest of pulsation. The salt solution was then washed off as quickly as possible, with the result that the pulsations slowly revived. Five minutes after the removal of the salt the recovery was partial, as seen in the successive pulsations, which now occurred at intervals of about 2 seconds, the post-diastolic pause being greatly prolonged. The recovery was more complete 15 minutes after the removal of the salt, the frequency being now 60 per minute.

It is seen that not only is the moto-excitability to external stimulation arrested by the application of the salt, but automatic cardiac pulsation is also arrested by the application of the same agent. It is to be noted that the pulsatory activity of the heart undergoes a revival after the removal of the salt, provided the application had not been unduly prolonged.

Thus, in both heart- and body-muscles, paralysis is induced by the direct localised action of salt on the tissue.

EFFECT OF SALT ON RHYTHMIC PULSATION OF LEAFLET OF *DESMODIUM*

The next investigation was carried out on plants with the view of finding out whether the characteristic action of

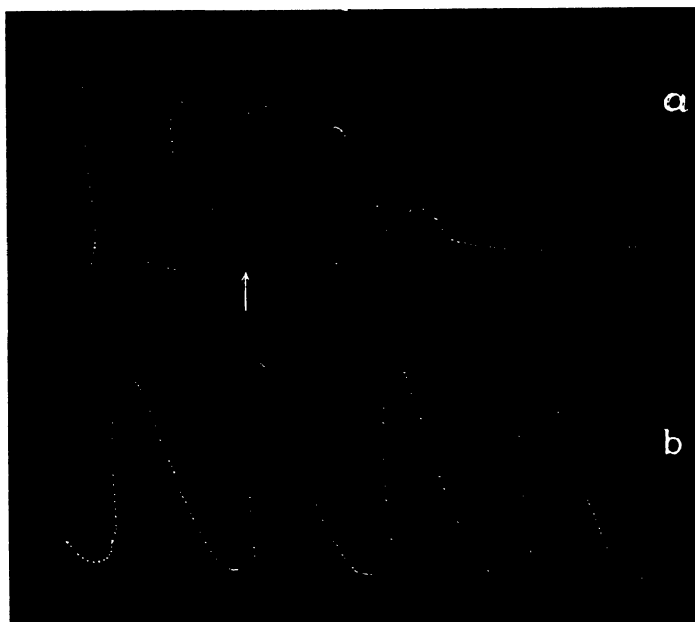


FIG. 41. Effect of application of salt on rhythmic pulsation of leaflet of *Desmodium gyrans*.

- a. Gradual arrest of pulsation after application of salt at arrow.
b. Quick removal of salt followed by revival in the course of 30 minutes.

salt is confined to the cardiac pulsation of the animal, or whether it is of wider application to rhythmic pulsations in general, including those exhibited by the lateral leaflet of the plant *Desmodium gyrans*. The pulsations of this plant have already been shown to exhibit characteristics similar to those of the automatic pulsations of the animal.¹

In order to obtain a record of the pulsations of the leaflet of *Desmodium*, it is attached, by means of a cocoon thread, to the arm of the magnifying lever of an Oscillating Recorder. The plate of this recorder oscillates to and fro at intervals of 3 seconds, thus making a dotted record. The period of a single oscillation in the plant is relatively long.

Experiment 72. *Effect of salt on pulsations of Desmodium*.—After taking record of a series of uniform pulsations strong solution of salt was applied at the arrow (fig. 41 (a)). Its effect was manifested in the course of 3 minutes, when the pulsation became continuously enfeebled, and came to a stop after 8 minutes. Without the least delay the salt was next washed off, after which the rhythmic activity became slowly revived. The revival was almost complete after 30 minutes.

SUMMARY

Many scaleless fishes are provided with sharp-pointed pectoral fins with which they inflict dangerous wounds when handled.

Application of salt on the body of the fish induces a motor-paralysis on account of which the fish is unable to strike or make any movement.

It is the absorption of the salt by the skin that induces the paralysis which spreads from point to point at a slow rate. When the salt is applied on the neck, the paralysis extends to both the flanks at the same rate, and the whole length of the fish is rendered inert in the course of about 5 minutes. The fish is then insensitive to either mechanical or chemical stimulation.

Asymmetrical application of salt on one flank of the fish

¹ Bose, *Irritability of Plants* (1913).

induces earlier paralysis of the proximal than of the distal side. The period of transmission from one side to the other varies from 4 to 6 minutes.

The induced paralysis by the application of salt is not permanent. On washing off the salt the fish fully regains its moto-excitability in the course of a few hours.

External application of salt does not affect the respiratory process nor the pulsation of the heart. On rendering the fish inert by application of salt, it is possible to study the cardiac activity under conditions which closely approximate to the normal.

The pulsation, recorded under such ideal conditions, remains perfectly uniform for many hours; this method thus offers a great advantage in investigations on the physiological reaction of the heart under external agencies.

Not only is the moto-excitability to external stimulation arrested by application of salt, but the automatic pulsation of the heart is also arrested by the application of the same agent. This is equally true of the cardiac pulsation of the fish and that of the frog. Provided the application has not been unduly prolonged, the removal of the salt is followed by a renewal of pulsatory activity.

Application of salt also arrests the rhythmic pulsation of the leaflet of *Desmodium gyrans*. Quick removal of salt is generally attended by revival of the pulsation.

The similarity of the effect on rhythmic pulsations in the animal and in the plant offers further evidence of the unity of their physiological mechanism.

IV.—CONDUCTION OF EXCITATION ALONG DEFINITE CHANNELS FROM THE DIFFERENT QUADRANTS OF PULVINUS OF *MIMOSA* TO THE PERIPHERY

BY

B. K. DUTT, B.Sc.

A LONG series of investigations, which prove that the transmission of excitation in plants is essentially similar to that of the nervous impulse in animals, has already been described.¹ When the nerve of an animal is stimulated in any way, an invisible impulse is transmitted to a distance which, impinging on the terminal motor organ, the muscle, causes a mechanical movement. In the sensitive plant *Mimosa pudica* a stimulus, applied on the stem or the petiole, also gives rise to an invisible impulse which is conducted to the distant motile organ, the pulvinus, causing a sudden fall of the leaf. A considerable similarity thus exists between the two impulses in the animal and in the plant. It has been suggested, that the responsive fall of the leaf is not due to the transmission of an excitatory impulse, but is brought about by a movement of sap. This theory is based on the supposition that a hypothetical stimulant, excreted as the result of irritation of the wood by a deep wound, is carried to the leaves by the transpiration current.

The fact that, without causing any deep wound, diverse modes of stimulation of the stem give rise to impulses which travel *simultaneously* both upwards and downwards, one in the direction of the movement of sap, the other against it, is sufficient to disprove the theory that the transpiration

¹ Bose, *Nervous Mechanism of Plants* (1926).

current is concerned in the transmission of the impulse. The process of transmission would thus appear to be a travelling of protoplasmic excitation.

The demonstration of the unity of conducting mechanism in the plant and in the animal is of such importance, that I undertook to repeat experiments with numerous species of sensitive plants under various climatic conditions in different provinces of India. My attempts in this direction proved to be invariably successful. This, as well as other confirmatory evidence obtained by independent methods of investigation, devised for the purpose, are described in the 'Transactions of the Bose Research Institute,' vol. vi, 1930-31.

INNERVATION OF THE FOUR QUADRANTS

Anatomical examination shows that the petiole has four main vascular bundles, containing as many phloem strands which function as conducting tissues and which converge towards the pulvinus. At the junction of the petiole and pulvinus the four strands are distinct, but in the pulvinus itself they appear to coalesce into an almost continuous ring. Each one of the phloem strands, as already stated, functions as a conducting tissue ; the left quadrant (1) is regarded as being connected with the sub-petiole (1) ; the right quadrant (4) with the sub-petiole (4) ; the lower quadrant (2) with the sub-petiole (2) ; and the upper quadrant (3) with the sub-petiole (3). The sub-petioles are consecutively numbered from left to right, the observer being supposed to face the central stem (fig. 42).

What fresh evidence can be brought forward to justify the above conclusion ?

EXPERIMENTAL DEMONSTRATION OF CONDUCTING COMMUNICATION

For facility of explanation, the terminations of the conducting tissue in the four quadrants will be distinguished as the central, and those in the sub-petioles as the peripheral

ends. If now there is a definite conducting communication between each quadrant and the corresponding sub-petiole, the fact should be capable of physiological demonstration by observing the effect of transmitted excitation from the centre to the periphery. The method of experimentation consists in initiating an excitatory impulse by local stimulation of each quadrant. The transmitted excitation is then detected

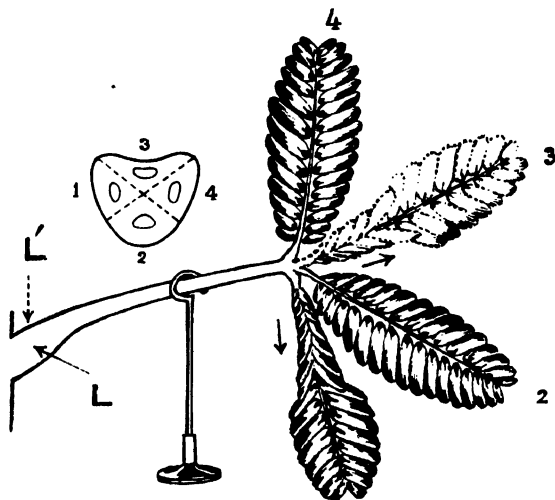


FIG. 42. Demonstration of physiological conduction between different quadrants and corresponding sub-petioles.

Diagrammatic representation of the four quadrants specially numbered is given in upper figure.

Stimulation of left quadrant or flank (1) by light L, causes responsive closure of the leaflets of sub-petiole (1). Stimulation of upper quadrant (3) by light L' gives rise to responsive closure of leaflets of sub-petiole (3).

at the periphery by the mechanical response of the motile indicators, which are the sensitive leaflets borne by each sub-petiole.

In order to establish the universality of the phenomenon, it is necessary to observe the effect of not merely a single, but of diverse modes of local stimulation at the different quadrants. These different modes are :

- (1) Photic stimulation.
- (2) Stimulation by electro-thermic radiation.
- (3) Mechanical stimulation.
- (4) Stimulation by constant electric current.

PHOTIC STIMULATION OF THE DIFFERENT QUADRANTS

Experiment 73. *Effect of photic stimulation.*—Stimulation of different quadrants is effected by the local application of a narrow beam of strong light from an arc lamp (*cf.* fig. 46, given in next chapter). The excitation, percolating inwards, reaches the central end of the conducting tissue and gives rise to an outgoing excitatory impulse which, reaching the corresponding sub-petiole, causes responsive closure of its leaflets. The stimulation of the sub-petiole is then regarded as *Indirect*, being due to transmitted excitation. If the stimulation be excessive, it becomes internally diffused and may produce a sudden fall of the whole leaf. But this does not modify the characteristic response of the leaflets of the particular sub-petiole. In order to prevent distraction during observation, the leaf of the petiole may be held in a supporting stand as in fig. 42.

Application of light on the left flank or quadrant (1) is found to cause a centrifugal impulse which, reaching the periphery, brings about successive closure of the leaflets of the sub-petiole (1). The right flank or quadrant (4) is next stimulated by light. The transmitted outgoing or centrifugal impulse now causes serial closure of the leaflets of the sub-petiole (4). Stimulation of the lower quadrant (2) by light gives rise to an impulse which causes the response of the leaflets of the sub-petiole (2). Finally, photic stimulation of the upper quadrant (3) transmits an impulse which results in the responsive closure of the leaflets of sub-petiole (3).

STIMULATION OF THE QUADRANTS BY ELECTRO-THERMIC RADIATION

Experiment 74. *Effect of thermic radiation.*—The passage of an electric current through a V-shaped piece of platinum

wire heats it short of incandescence (*cf.* fig. 49). Local stimulation can thus be produced by electro-thermic radiation. The effects are similar to those brought about by light. Local stimulation at quadrant (1) and at quadrant (4) give rise to centrifugal impulses, which cause closure of leaflets in sub-petiole (1) and (4) respectively. Stimulation at quadrants (3) and (2) induce responsive closure of leaflets of corresponding sub-petiole (3) and of sub-petiole (2).

MECHANICAL STIMULATION OF THE DIFFERENT QUADRANTS

The following is an interesting method of producing local stimulation of the different quadrants. The petiole is held in its normal position in a clamp, and a fine sharp pin is forced into one or the other of the quadrants till the conducting tissue is reached. The prick gives rise to intense stimulation which, becoming diffused, causes responsive closure of the leaflets of all sub-petioles. There is, however, a complete recovery after a suitable period of rest. For local stimulation, each quadrant should be subjected to moderate stimulation, which is effected by rubbing the head of the pin with a brush. The vibration thus produced reaches the central end of the conducting tissue and gives rise to an outgoing excitatory impulse which is transmitted to the corresponding sub-petiole.

It is to be borne in mind that in the following experiments the term 'stimulation of a quadrant' indicates stimulation of the central end of the conducting tissue in that quadrant.

Experiment 75. *Effect of mechanical stimulation.*—The left quadrant (1) of the pulvinus was first mechanically stimulated; this produced closure of the leaflets of sub-petiole (1). The right quadrant (4) was then subjected to similar stimulation; this resulted in the closure of leaflets of sub-petiole (4). On stimulation of the upper quadrant (3) the leaflets of sub-petiole (3) exhibited the movement of closure. Finally stimulation of lower quadrant (2) brought about the responsive closure of the leaflets of sub-petiole (2).

STIMULATION OF THE QUADRANTS BY POLAR ACTION
OF CONSTANT CURRENT

In the animal nerve excitation is initiated at kathode by the make of a constant current. In his 'Plant Response'¹ Sir J. C. Bose has proved that in the undifferentiated protoplasm of the plant body the polar effect of a constant current is identical with that in the animal. In both it is the sudden make of a constant current that initiates excitation at the kathodic point.

For the purpose of the following experiments the excitation of any quadrant can be initiated by making suitable electric connections so that the particular quadrant may become kathode. The excitation, it is to be remembered, occurs only by the sudden starting of the constant current from a voltaic battery.

The electromotive force required can be tapped off from a thirty-volt battery of dry cells. The starting of the constant current for excitation is effected at the proper time by means of a tapping key.

For making electric connections with the plant, the wire from the positive end of the battery is wound round the stem and good electrolytic contact secured by wrapping a piece of moist cloth round the wire. This is made with an indifferent point in the stem about 1 cm. below the pulvinus.

The other electrode, which is to be rendered the kathode by the starting of the current, consists of a pin-applicator to be pricked into the quadrant. Less injury to the tissue is caused by a sharp-pointed pin, and in practice an enamel-coated pin, having only the prick point exposed, gives satisfactory results. The pin-applicator connected with the negative pole of the battery is thrust into the quadrant of the pulvinus so as just to reach the central end of the conducting tissue.

This process of thrusting in the pin, as previously stated, gives rise to intense wound stimulation which becomes diffused. But after half an hour's rest there is a recovery.

¹ Bose, *Plant Response* (1906), p. 199.

Local stimulation of each quadrant can now be effected by the sudden starting of the current, the pin-applicator being the kathode.

The following is the method of procedure. A voltaic battery V, giving the necessary electromotive force, is taken and its positive terminal A connected with the indifferent

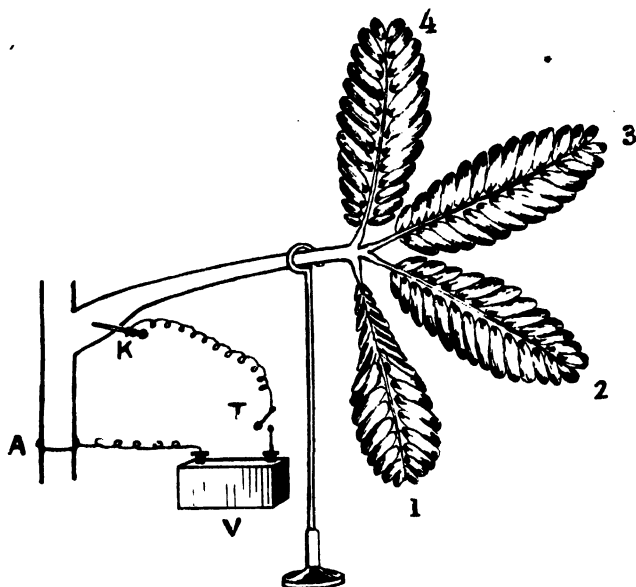


FIG. 43. Representation of method of polar excitation initiated at different quadrants.

v, battery of cells; T, tapping key for starting current; A, indifferent connection on stem; K, pin-applicator which is applied to the left quadrant (1). Kathodic excitation at K gives rise to centrifugal impulse bringing about closure of leaflets in sub-petiole (1) (*see text*).

point in the stem. The pin-applicator is suitably applied at a particular quadrant. After recovery from the effect of the pin-prick, excitation is initiated at the quadrant on the sudden starting of the current by the tapping-key T, K being made the kathode. The subject will be understood by reference to fig. 43, in which K is applied at the left quadrant. The results of experiments carried out with electric stimulation at different quadrants are given below.

CONDUCTION OF EXCITATION ALON

Experiment 76. *Effect of local dividial quadrant.*—The results of stimulation of different quadrants are as follows :

(a) *Stimulation of left flank or quadrant* (1).—The current, stimulation of the central end of t.

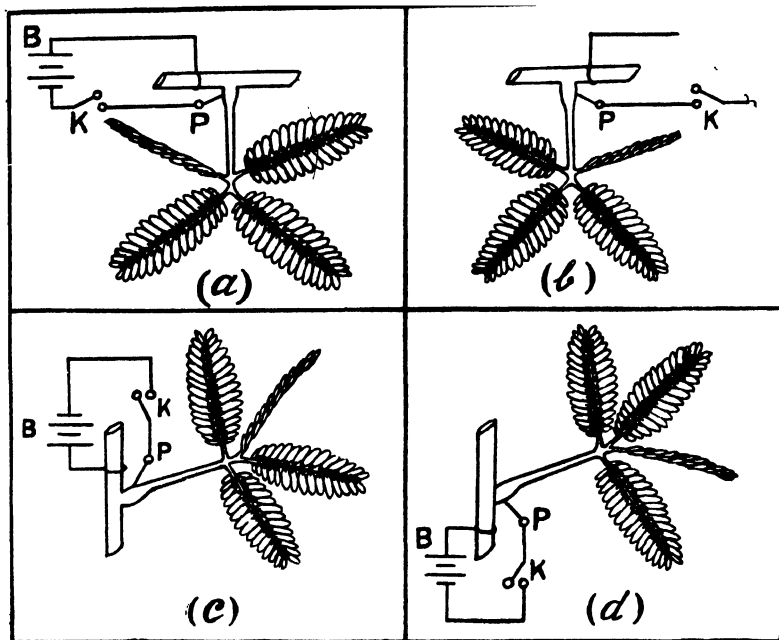


FIG. 44. Diagrammatic representation of effects of local stimulation of different quadrants on response of leaflets of sub-petioles (see text).

tissue of quadrant (1) was initiated. After a short time the transmitted outgoing or centrifugal impulse reached the sub-petiole (1) and caused the closure of its leaflets (fig. 43).

(b) *Stimulation of right flank or quadrant* (4).—Kathodic stimulation in this case gave rise to a centrifugal impulse, which caused closure of leaflets of sub-petiole (4).

(c) *Stimulation of upper quadrant* (3).—The centrifugal impulse initiated by the kathode-make brought about the closure of leaflets of sub-petiole (3).

lower quadrant (2).—The result of the stimulation was the initiation of a centrifugal induced closure of leaflets of sub-petiole (2). The results are diagrammatically represented in

It is interesting to note that best results are secured when the applicator is applied on points of quadrants close to the petiole and away from the stem. The explanation of this peculiarity is found in the fact that the four quadrants are distinct from each other towards the petiole, whereas they coalesce into an almost continuous ring towards the stem.

The following tabular statement summarises the characteristic effects obtained in three typical series of observation. It is to be borne in mind that the electrical resistance offered by different specimens was not the same. Hence, the intensity of current, in micro-amperes, that flowed through the circuit, was not the same in all cases.

TABLE IX.—TRANSMITTED EFFECTS DUE TO POLAR STIMULATION OF QUADRANTS

Series of observation	Stimulation at quadrant	Voltage applied	Intensity of current	Responsive closure of leaflets in
I.	Quadrant (1)	16 volts	28 micro-amps.	Sub-petiole (1)
	" (4)	16 "	27 "	" (4)
	" (3)	12 "	24 "	" (3)
	" (2)	14 "	27 "	" (2)
II.	Quadrant (1)	20 volts	38 micro-amps.	Sub-petiole (1)
	" (4)	18 "	32 "	" (4)
	" (3)	20 "	42 "	" (3)
	" (2)	16 "	26 "	" (2)
III.	Quadrant (1)	18 volts	35 micro-amps.	Sub-petiole (1)
	" (4)	16 "	32 "	" (4)
	" (3)	20 "	45 "	" (3)
	" (2)	14 "	26 "	" (2)

CONDUCTION OF EXCITATION ALONG

SUMMARY

A centrifugal or outgoing impulse is g.
stimulation of the central end of the conduc
each quadrant of the pulvinus.

The transmitted impulse causes the mechanical
of the leaflets of the corresponding sub-petiole only.
experiments prove that : quadrant (1) is in conduct.
communication with sub-petiole (1) ; quadrant (4) with
sub-petiole (4) ; quadrant (3) with sub-petiole (3) ; and
quadrant (2) with sub-petiole (2).

The universality of the phenomenon is established by
the application, not only of one particular, but of all modes
of stimulation, such as photic, electro-thermic, mechanical,
as well as polar stimulation by constant current.

44

ADDITIVE AND DIFFERENTIAL ACTION OF CENTRAL AND PERIPHERAL STIMULATION ON THE RESPONSE OF THE PULVINUS

BY

B. K. DUTT, B.Sc.

LOCAL stimulation of the four quadrants of the pulvinus of *Mimosa* has, in the last chapter, been shown to initiate centrifugal impulses, which, reaching the sub-petioles, cause characteristic responsive closure of their leaflets. The definite results thus obtained demonstrate that a conducting communication exists between each quadrant and its corresponding sub-petiole. This conclusion will find strong and independent support if it can be shown that peripheral stimulation of a sub-petiole gives rise to an ingoing or centripetal impulse which, reaching the corresponding quadrant, causes a response characteristic of that quadrant.

The responding organ at the periphery is the motile leaflet of the sub-petiole which, by its movement, indicates the arrival of the outgoing or centrifugal impulse initiated at a particular quadrant of the pulvinus. The question next arises: How is the ingoing centripetal impulse, generated at the periphery by stimulation of the sub-petiole, to be detected by the responsive reaction of the corresponding quadrant? This is only possible if each quadrant gives a response characteristic of that quadrant.

These characteristic reactions can be discovered by finding the effect of direct stimulation of each quadrant, not only under one, but under diverse modes of stimulation. The results of this inquiry have been given elsewhere.¹ Additional experiments which I have been able to carry

¹ Bose, *Nervous Mechanism of Plants*, 1926.

ADDITIVE AND DIFFERENTIAL

out on the subject will be described in this Paper.

After the determination of the character of each quadrant, experiments will be described of ingoing or centripetal impulse at each of the quadrants, reaching the centre, causes the characteristic of the corresponding quadrant. This will obviously be an independent proof of the conducting communication existing between the periphery and the centre.

I have been able to devise two new methods of inquiry which will be found to yield very important results. These are the *Additive* and the *Differential Effects* of direct and indirect stimulations.

The investigations in this chapter will be described in the following order :

- I. The effects of *Direct* stimulation of different quadrants.
- II. The effects of *Indirect* stimulation of the quadrants.
- III. *Additive* effect of *Direct* and *Indirect* stimulation.
- IV. *Differential* effect of *Direct* and *Indirect* stimulation.

TORSIONAL RESPONSE

The quadrants of pulvinus, when directly stimulated, exhibit four distinct types of response. These characteristic responses, rectilinear or torsional, are determined by the particular quadrant that is subjected to stimulation. The rectilinear responses are either up or down ; while the torsional responses are clockwise or anti-clockwise.

The record of torsional movement is obtained by the Torsional Recorder. For elimination of the effect of the weight of the leaf, and for obtaining record of pure torsion, the petiole is held in a hooked glass support with a smooth internal surface, thus allowing freedom for torsional response. The torsion is magnified by an L-shaped piece of aluminium wire, so tied to the petiole that its free arm is at right angles to the former. The free end of the aluminium wire is connected by a silk thread to the short arm of a recording lever,

and magnification of the torsional
handed or anti-clockwise torsion pro-
e in the record, and a right-handed or
on produces a down-curve. The record is

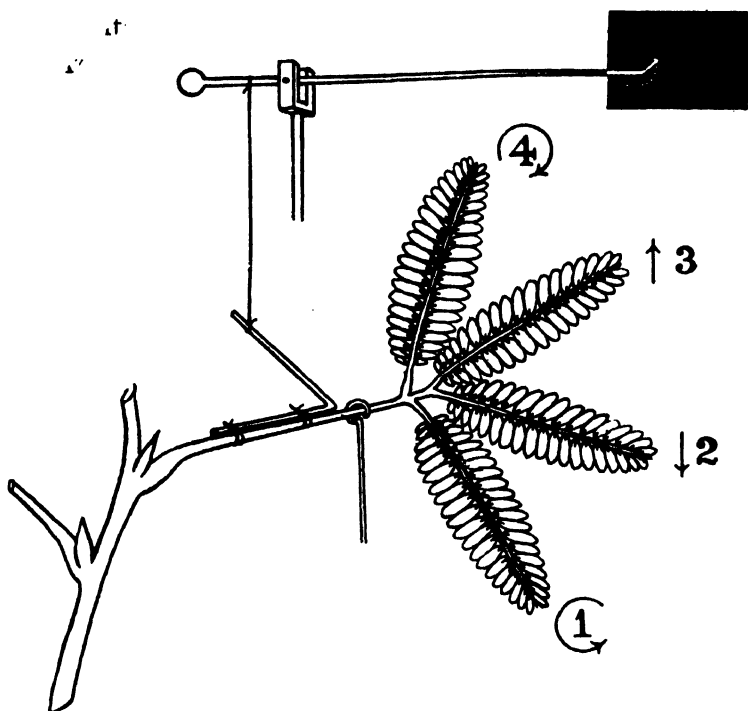


FIG. 45. Recorder for Torsional Response.
Stimulation of left quadrant (1) induces anti-clockwise, that of
the right quadrant (4), a clockwise torsion. Stimulations
of quadrants (2) and (3) induce rectilinear down- and up-
movements respectively.

taken on an oscillating smoked-glass plate, the successive
dots being at definite intervals of time (fig. 45). The same
apparatus can be used for obtaining rectilinear up- and
down-records; the hooked support is then removed and
the short arm of the lever directly attached by a thread to
the petiole.

ADDITIVE AND DIFFERENTIAL

THE EFFECTS OF DIRECT STIMULATION

The quadrants are subjected to direct stimulation, such as photic and radio-thermia.

PHOTIC STIMULATION

The source of light is a self-feeding arc-lamp by which light of constant intensity can be maintained. The objective lens of the lantern gives a horizontal beam of light

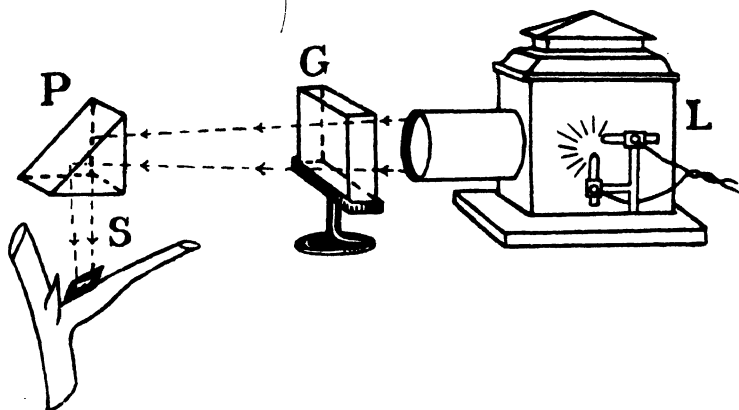


FIG. 46. Local application of photic stimulus on different quadrants.

L, arc-lamp; G, trough of glass containing alum solution for absorption of heat rays; P, right-angled prism for directing light on upper or lower quadrants; S, piece of black paper with narrow slit placed on quadrant.

which is slightly convergent. In front of this lens is a parallel-sided glass trough filled with alum solution for absorption of the heat rays. For the stimulation of the upper and lower quadrants, the light from the arc-lamp is totally reflected by a right-angled prism, upwards or downwards, as the case may be. To prevent the light diffusing over neighbouring quadrants, a small piece of black paper with a narrow slit is attached to the quadrant to be stimulated (fig. 46). For the lateral stimulation of

B. K. DUTT

as no glass prism is necessary, the
 shown directly on the pulvinus through

77. *Effect of photic stimulation of left*
 When light was thrown laterally on the left
 anti-clockwise torsion was induced, recorded

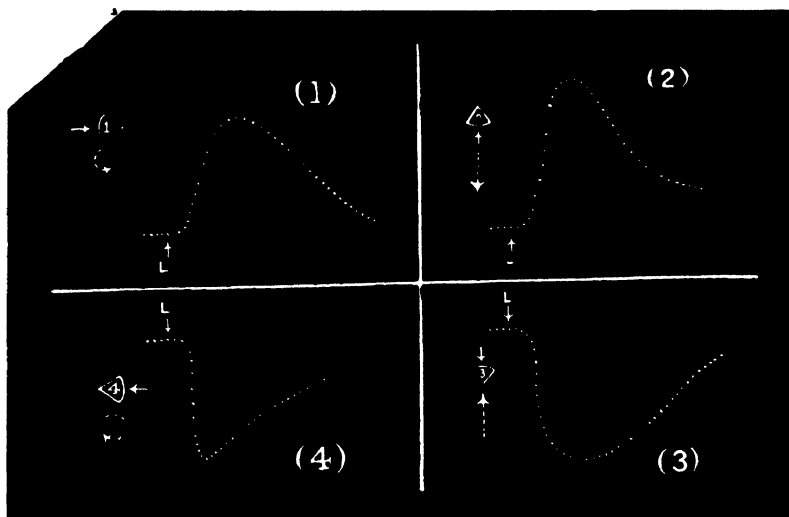


FIG. 47. Records of effect of direct photic stimulation on different quadrants.

In semi-diagrammatic representation seen to the left of each figure, the thick arrows indicate direction of incident stimulus at different quadrants. The dotted circular or straight arrows indicate the actual direction of responsive movements. In (1) and (4) are shown the anti-clockwise and clockwise torsions of quadrants (1) and (4). The curves in (2) and (3) exhibit rectilinear down- and up-responses of quadrants (2) and (3).

as an up-curve. There was a recovery on the cessation of light (fig. 47 (1)).

Experiment 78. *Effect of stimulation of right quadrant (4).*—The direction of the incident light was changed, the right quadrant being subjected to stimulation. The response was now by a clockwise torsion, seen as a down-curve (fig. 47 (4)).

Experiment 79. *Effect of stimulation of lower quadrant*

(2).—As the responses in this and in the following experiment are not torsional but rectilinear, the glass hook supporting the leaf was removed, and the petiole directly attached to the short arm of the recording lever. On stimulation of the lower quadrant the response was a down-movement of the petiole, represented by an up-curve (fig. 47 (2)).

Experiment 80. *Effect of stimulation of upper quadrant*

(3).—On stimulation of the upper quadrant, the response was a rectilinear up-movement, represented by a down-curve (fig. 47 (3)).

The results are diagrammatically represented in fig. 48.

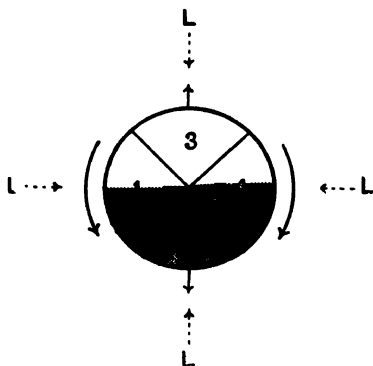


FIG. 48. The characteristic responses of different quadrants.

Direction of incident light represented by dotted arrow; full arrow represents responsive movement. Lateral stimulation induces anti-clockwise and clockwise torsions; light incident from above or below induces rectilinear movements. Lower half of pulvinus shaded.

RADIO-THERMIC STIMULATION

The thermal radiation for stimulation is produced by an Electro-thermal Radiator. This consists of a V-shaped loop of platinum wire heated short of incandescence by the passage of an electric current. The point V of the radiator is held very near the quadrant of the pulvinus which is to be stimulated. The electric radiator is in circuit with a storage battery of 6 volts, as well as with a tapping key and a metronome interrupter. This latter is so adjusted

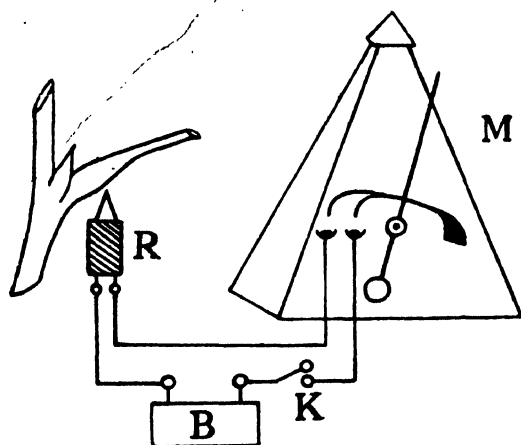


FIG. 49. The Electro-thermal Radiator.

V-shaped platinum loop projecting from R is maintained short of incandescence by passage of current from battery B. The tapping key K being kept pressed down, duration of application of stimulus is adjusted by metronome M.

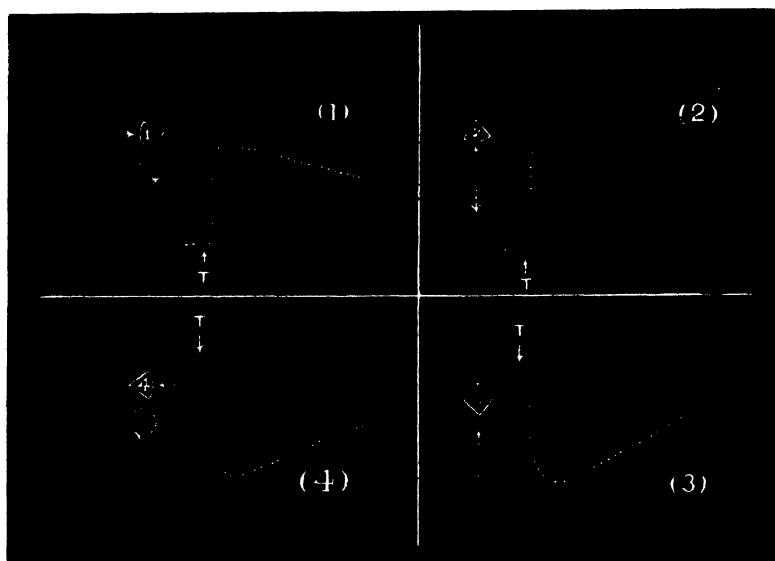


FIG. 50. Effect of thermal radiation on different quadrants. Anti-clockwise and clockwise torsions of quadrants (1) and (4). Rectilinear down- and up-responses of quadrants (2) and (3). Compare results with those given in fig. 47.

that at every alternate tick it completes the electric circuit. The radiation remaining constant, the intensity of stimulation depends on the duration of application; this can be kept constant by counting a definite number of ticks of the metronome, during which period the key remains pressed down (fig. 49).

Experiment 81. *Effects of radio-thermic stimulation on left (1) and on right (4) quadrants.*—Stimulus of thermal radiation is found to induce effects precisely similar to those of photic stimulus. When the left quadrant was stimulated by thermal rays, the response is by an anti-clockwise torsion; stimulation of the right quadrant gives rise, on the other hand, to a clockwise torsion (fig. 50 (1), (4)).

Experiment 82. *Effects of radio-thermic stimulation on lower (2) and on upper (3) quadrants.*—As under photic stimulation, so also under radio-thermic stimulation, the lower quadrant responds by a rectilinear down- and the upper quadrant by a rectilinear up-movement (fig. 50 (2), (3)).

The effects of direct application of stimulus are summarised below.

TABLE X.—EFFECTS OF DIRECT STIMULATION OF QUADRANTS BY PHOTIC AND BY RADIO-THERMAL STIMULUS

Stimulation	Quadrant	Response
Photic,	Left (1)	Anti-clockwise movement
as	Right (4)	Clockwise movement
also	Lower (2)	Down-movement
Thermal	Upper (3)	Up-movement

EFFECT OF INDIRECT STIMULATION OF THE QUADRANTS

A responding organ can be stimulated not only *directly*—*i.e.* by stimulus applied on the organ itself—but also *indirectly* by excitation transmitted from a distance. Thus a muscle exhibits a mechanical response when it is stimulated not only directly but also indirectly by the excitatory

impulse transmitted from a distance along a conducting nerve. Similarly, each quadrant of the pulvinus exhibits its characteristic response, not only under stimulus directly applied on it, but also under the transmitted effect of stimulus which had been applied on a distant sub-petiole, *provided the quadrant and the particular sub-petiole are in conducting communication with each other.*

The four sub-petioles, in each of which the excitatory impulse is initiated for indirect stimulation of the quadrants, will be distinguished as S_1 , S_2 , S_3 , and S_4 , counted from left to right (*cf.* fig. 45).

PHOTIC STIMULATION

Experiment 83. *Effect of indirect stimulation of quadrant by photic stimulation of sub-petiole S_1 .*—A horizontal beam of strong light from the arc-lamp lantern is thrown down on the sub-petiole by means of a totally reflecting prism. Since the impulse generated is enfeebled by transmission to a distance, a somewhat prolonged exposure of strong light is necessary for initiation of minimally effective excitation. The transmitted impulse was found to reach the pulvinus after a short time, and to induce an anti-clockwise torsion (fig. 51₍₁₎), this being the characteristic response of quadrant (1).

Experiment 84. *Effect of indirect stimulation of quadrant by photic stimulation of sub-petiole S_4 .*—The transmitted effect of this induced a clockwise torsion (fig. 51₍₄₎), which is the characteristic response of quadrant (4) under direct stimulation.

Experiment 85. *Effect of indirect stimulation of quadrant by photic stimulation of sub-petiole S_2 .*—This gave rise to a rectilinear down-movement (fig. 51₍₂₎), which is the characteristic response of quadrant (2).

Experiment 86. *Effect of indirect stimulation of quadrant by photic stimulation of sub-petiole S_3 .*—The effect of stimulation of this sub-petiole was an impulse which caused a rectilinear up-movement (fig. 51₍₃₎), this being the characteristic response of quadrant (3).

By means of centrifugal impulse initiated at the centre it has been shown in the previous chapter (*cf.* Experiments 73, 74) that each quadrant of the pulvinus is in conducting communication with its corresponding sub-petiole. Now the characteristic effects of centripetal impulse described

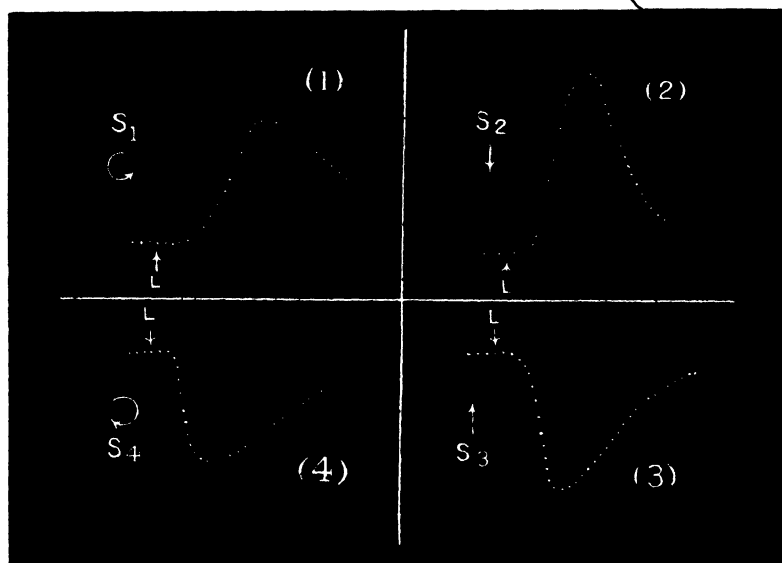


FIG. 51. Response of four quadrants of pulvinus to indirect photic stimulation.

In this and in following records of effect due to indirect stimulation, the circular and straight arrows at S_1 , S_4 , S_2 , S_3 , indicate the actual directions of the responsive movements.

Stimulation of sub-petioles S_1 and S_4 induced anti-clockwise and clockwise torsions respectively. Stimulation of sub-petioles S_2 and S_3 induced rectilinear down- and up-movements.

above offer crucial and independent proof that *each sub-petiole is in conducting communication with its corresponding quadrant*.

In order to demonstrate the universality of the phenomenon, additional experiments are described on the effect of indirect stimulation induced by thermal radiation.

RADIO-THERMIC STIMULATION

Experiment 87. *Effects of indirect stimulation of quadrants by radio-thermic stimulation of sub-petioles.*—The characteristic results are precisely similar to those under indirect photic stimulation—that is to say, stimulation of sub-petioles S_1 and S_4 induced anti-clockwise and clockwise torsions respectively; stimulation of sub-petioles S_2 and S_3 , on the other hand, gave rise respectively to rectilinear down- and up-responses.

Hitherto the stimulus employed had been either photic or thermal. It occurred to me that an altogether different mode of indirect stimulation might with advantage be employed—namely, that due to tetanising shocks from an induction coil.

ELECTRIC STIMULATION

The sub-petiole in which the transmitted impulse is to be initiated is put in series with the secondary of an induction coil, the intensity of the induction shock for minimally effective stimulation being regulated by the gradual approach of the secondary coil towards the primary. A tapping key and a metronome are interposed in series with the primary coil. The duration of the application of stimulus can thus be maintained constant for successive experiments. The effectiveness of electric stimulation by tetanising induction shocks is found to be very pronounced; in fact, the minimally effective intensity of the shock was often found to be as low as 0.4 unit on an arbitrary scale.

Experiment 88. *Effect of indirect stimulation of quadrant by electric stimulation of S_1 .*—The transmitted effect of electric stimulation induced an anti-clockwise torsion (fig. 52₍₁₎), characteristic of the response of quadrant (1).

Experiment 89. *Effect of indirect stimulation of quadrant by electric stimulation of S_4 .*—This induced a clockwise torsion (fig. 52₍₄₎), characteristic of the response of quadrant (4).

Experiment 90. *Effect of indirect stimulation of quadrant*

by electric stimulation of S_2 .—A rectilinear down-movement was induced (fig. 52 (2)), which was the characteristic response of quadrant (2).

Experiment 91. *Effect of indirect stimulation of quadrant by electric stimulation of S_3 .*—This caused a rectilinear up-

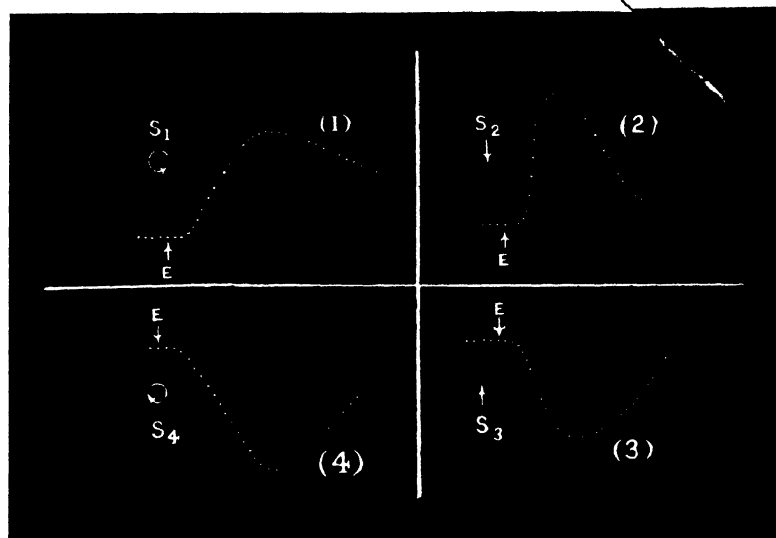


FIG. 52. Response of four quadrants of pulvinus to indirect electric stimulation (*cf.* fig. 51).

movement (fig. 52 (3)), the characteristic response of quadrant (3).

The effects of indirect stimulation under diverse forms of stimulus are given below.

TABLE XI.—EFFECT OF INDIRECT STIMULATION.

Stimulation	Sub-petiole	Characteristic response	Of quadrant
Photic, Thermal, and Electric	(1)	Anti-clockwise movement	(1)
	(4)	Clockwise movement	(4)
	(2)	Down-movement	(2)
	(3)	Up-movement	(3)

The results of the investigations described may now be briefly recapitulated, and the effects of direct stimulation compared with those of indirect stimulation.

- i. Direct stimulation of the left quadrant (1) induces an anti-clockwise torsion; the effect of indirect stimulation transmitted from sub-petiole (1) produces the same result.
- ii. Direct stimulation of the lower quadrant (2) induces a rectilinear down-movement; similar result is

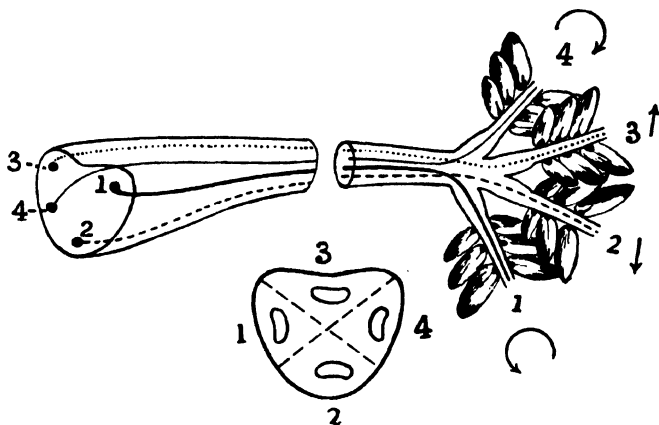


FIG. 53. Showing the course of four conducting strands from four sub-petioles to the quadrants of the pulvinus.

The characteristic responsive movement of the leaf under indirect stimulation of the four sub-petioles are represented by circular and rectilinear arrows.

The lower figure is a diagrammatic section of the quadrants.

produced by the effect of indirect stimulation transmitted from sub-petiole (2).

- iii. Direct stimulation of the upper quadrant (3) induces a rectilinear up-movement; the same result occurs by the effect of indirect stimulation from sub-petiole (3).
- iv. Direct stimulation of the right quadrant (4) induces a clockwise torsion; the effect of indirect stimulation transmitted from sub-petiole (4) produces the same result.

ADDITIVE AND DIFFE.

The above facts prove conclu-
definite conducting communication
petiole and its corresponding quadra-
The conducting connections between the sul-
four quadrants are diagrammatically represente.

After demonstrating the individual effects o-
indirect stimulations, experiments will next be c-
how these individual effects could be made to act in
cordance or in opposition. The results thus obtained w-
be found to offer evidence of a crucial character in regard
to the specific conducting communication which exists
between the periphery and the centre.

THE ADDITIVE EFFECT OF INDIRECT AND DIRECT STIMULATION

In the following investigations the stimulus employed is
light, the responding organ being a particular quadrant of
the pulvinus. This quadrant is first stimulated indirectly—
that is to say, by stimulation of a distant sub-petiole ; this
indirect stimulation is indicated by the symbol Is , to which
the number of sub-petiole itself is added. While the re-
sponse of a particular quadrant to indirect stimulation is in
progress, direct stimulation of the same quadrant, sym-
bolised by Dq , is effected by direct incidence of light on it.
Both the indirect and direct stimulations were caused by light
from two arc-lamps after passage through parallel-sided
troughs filled with alum solution for cutting off the heat rays.

Experiment 92. *Additive effect of indirect stimulation of
quadrant (1) and of direct stimulation of the same quadrant.*—
It has been explained that the quadrant (1) is indirectly
stimulated by the excitation transmitted from the sub-
petiole (1). This indirect stimulation, conveniently desig-
nated as Is_1 , induced an anti-clockwise torsion of quadrant (1),
recorded as an up-curve. While this torsional response
was in progress, quadrant (1) was directly stimulated at the
moment indicated by Dq_1 shown by the horizontal arrow.
This resulted in a great enhancement of the rate of anti-
clockwise torsion, seen in the sudden erection of the curve.

and of peripheral stimulation was by (fig. 54).

Additive effect of indirect and of direct stimulation of quadrant (4).—Transmitted excitation Is_4 from (4) induced clockwise torsion of quadrant (4), recorded as a down-curve; direct stimulation Dq_4 of (4) at horizontal arrow greatly increased the rate

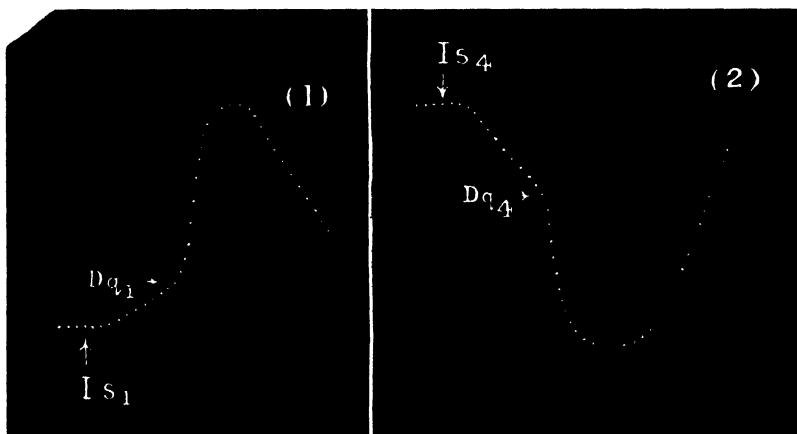


FIG. 54.

FIG. 55.

FIG. 54. Indirect stimulation Is_1 from sub-petiole (1) induced anti-clockwise torsion (up-curve). Direct stimulation Dq_1 of the first quadrant induced an enhancement of rate of torsion. Cessation of stimulation is followed by recovery in this and in the following cases.

FIG. 55. Indirect stimulation Is_4 from sub-petiole (4) induced clockwise torsion (down-curve). Direct stimulation Dq_4 of the fourth quadrant enhanced the rate of clockwise torsion.

of clockwise torsion (fig. 55). The usual recovery occurred on the cessation of stimulation.

Experiment 94. *Additive effect of indirect and of direct stimulation of quadrant (2).*—Transmitted excitation Is_2 from sub-petiole (2) induced a rectilinear down-movement of quadrant (2), recorded as an up-curve. Direct stimulation Dq_2 of the same quadrant at horizontal arrow induced a great increase in the rate of down-movement (fig. 56).

Experiment 95. *Additive effect of indirect and of direct stimulation of quadrant (3).*—Transmitted excitation Is_3 from sub-petiole (3) induced a rectilinear up-movement of quadrant (3), represented by a down-curve. Direct stimulation Dq_3 of the same quadrant at horizontal arrow caused a great increase in the rate of the up-movement (fig. 57).

These additive effects of indirect and direct stimulation can only be explained by the transmission of excitation

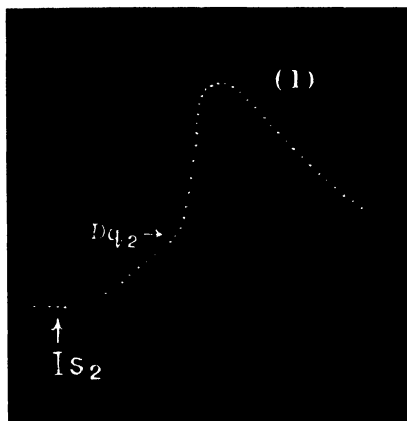


FIG. 56.

FIG. 56. Indirect stimulation Is_2 from sub-petiole (2) induced rectilinear down-movement (up-curve). Direct stimulation Dq_2 of the second quadrant enhanced rate of down-movement.

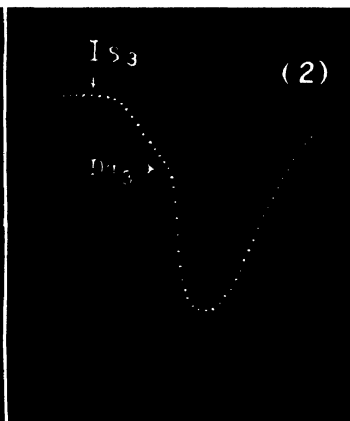


FIG. 57.

FIG. 57. Indirect stimulation Is_3 from sub-petiole (3) induced rectilinear up-movement (down-curve); direct stimulation Dq_3 of the third quadrant enhanced rate of up-movement.

along a definite conducting channel from a given petiole at the periphery to the corresponding quadrant at the centre. This will be clearly understood from the tabular statement on p. 116.

Employing the usual symbols, it is seen that—

Is_1	produces the same anti-clockwise response as	Dq_1 .
Is_4	„ „ clockwise „ „	Dq_4 .
Is_2	„ „ rectilinear down-response „	Dq_2 .
Is_3	„ „ „ up-response „	Dq_3 .

TABLE XII.—THE ADDITIVE EFFECT OF INDIRECT AND DIRECT STIMULATION.

Stimulation	Characteristic response of the quadrant	Resulting effect
Indirect (from sub-petiole 1) Direct (of quadrant 1)	Anti-clockwise torsion " "	Enhancement of the rate
Indirect (from sub-petiole 4) Direct (of quadrant 4)	Clockwise torsion " "	Enhancement of the rate
Indirect (from sub-petiole 2) Direct (of quadrant 2)	Rectilinear down-movement Rectilinear down-movement	Enhancement of the rate
Indirect (from sub-petiole 3) Direct (of quadrant 3)	Rectilinear up-movement Rectilinear up-movement	Enhancement of the rate

Since the effect of indirect is similar to that of direct stimulation, their combined effect must necessarily be additive. The experimental results described thus lead to the establishment of the following law :

When the effects of indirect and direct stimulation conspire with each other, then their resultant effect is additive.

DIFFERENTIAL EFFECT OF INDIRECT AND DIRECT STIMULATION

When concordant reactions of indirect and direct stimulation induce a resultant effect which is additive, then

antagonistic reactions must necessarily induce a differential effect.

It has been explained that the responses of opposite quadrants are antagonistic to each other. Thus, while stimulation of the left quadrant (1) induces an anti-clockwise torsion, that of the right quadrant (4) gives rise to a clockwise torsion. Further, while stimulation of the lower quadrant (2) induces a rectilinear down-movement, that of quadrant (3) causes a rectilinear up-movement.

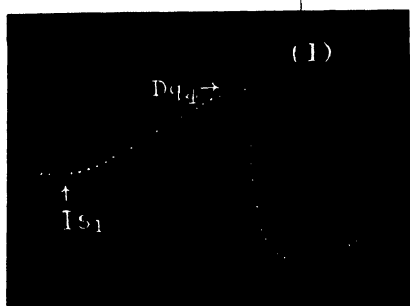


FIG. 58.

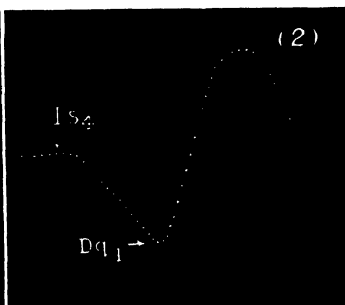


FIG. 59.

FIG. 58. Differential effect of indirect and direct stimulation. Indirect stimulation Is_1 induced anti-clockwise torsion of quadrant (1) (up-curve); direct stimulation Dq_4 of antagonistic quadrant arrested and reversed movement into clockwise torsion (down-curve).

FIG. 59. Indirect stimulation Is_4 induced clockwise torsion (down-curve), which was arrested and reversed by direct stimulation Dq_1 of antagonistic quadrant.

It would thus follow that the effect of indirect stimulation of each quadrant could be opposed by direct stimulation of the opposite quadrant. Since the effect of indirect stimulation is relatively feeble, on account of transmission from a distance, direct stimulation of the opposite quadrant would therefore be expected first to neutralise and then to cause a reversal of the response initiated by the indirect stimulation.

I proceed to show that these theoretical inferences are fully verified by results of experiments.

Experiment 96. *Reversal of response of quadrant (1), due to transmitted excitation from sub-petiole (1), by the direct stimulation of the antagonistic quadrant (4).—*Transmitted excitation IS_1 from sub-petiole (1) induced an anti-clockwise torsion of quadrant (1), represented by an up-curve. Direct

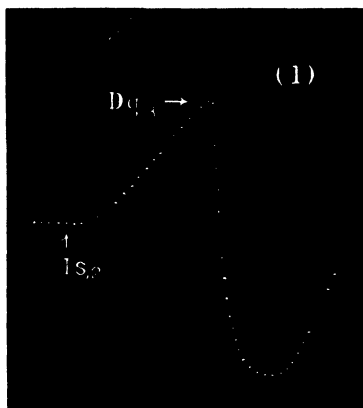


FIG. 60.

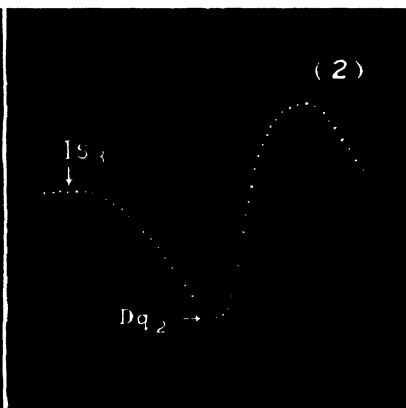


FIG. 61.

FIG. 60. Indirect stimulation IS_2 induced rectilinear down-movement (up-curve), which was arrested and reversed into up-movement (down-curve) by direct stimulation Dq_3 of antagonistic quadrant.

FIG. 61. Indirect stimulation IS_3 induced rectilinear up-movement (down-curve). This was arrested and reversed into down-movement (up-curve) by direct stimulation Dq_2 of antagonistic quadrant.

stimulation Dq_4 of antagonistic quadrant caused a quick arrest and reversal into clockwise torsion, shown by down-curve. On the cessation of stimulation there was a recovery (fig. 58).

Experiment 97. *Reversal of response of quadrant (4), due to transmitted excitation from sub-petiole (4), by the direct stimulation of the antagonistic quadrant (1).—*The transmitted excitation IS_4 from sub-petiole (4) induced a clockwise torsion, indicated by a down-curve. Direct stimulation Dq_1 of antagonistic quadrant (1) not only retarded the particular torsional response, but actually reversed it into an anti-clockwise torsion, as shown by the up-curve (fig. 59).

Experiment 98. *Reversal of response of quadrant (2), due to transmitted excitation from sub-petiole (2), by the direct stimulation of the antagonistic quadrant (3).*—The transmitted excitation Is_2 from sub-petiole (2) induced a

TABLE XIII.—SHOWING DIFFERENTIAL EFFECT OF INDIRECT AND DIRECT STIMULATION.

(Effect of direct stimulation given in italics.)

Stimulation	Individual response of the quadrant	Resulting effect
Indirect (from sub-petiole 1) Direct (of quadrant 4)	Anti-clockwise torsion <i>Clockwise torsion</i>	Clockwise torsion
Indirect (from sub-petiole 4) Direct (of quadrant 1)	Clockwise torsion <i>Anti-clockwise torsion</i>	Anti - clockwise torsion
Indirect (from sub-petiole 2) Direct (of quadrant 3)	Rectilinear down-movement <i>Rectilinear up-movement</i>	Movement upwards
Indirect (from sub-petiole 3) Direct (of quadrant 2)	Rectilinear up-movement <i>Rectilinear down-movement</i>	Movement downwards

rectilinear down-movement, represented by an up-curve. Direct stimulation Dq_3 of quadrant (3) at arrow first arrested the down-movement and subsequently reversed it into an up-movement, represented by down-curve (fig. 60).

Experiment 99. *Reversal of response of quadrant (3), due to transmitted excitation from sub-petiole (3), by the direct stimulation of the antagonistic quadrant (2).*—The

transmitted excitation Is_3 from sub-petiole (3) induced a rectilinear up-movement, indicated by a down-curve. Direct stimulation Dq_2 of quadrant (2) not only arrested this particular movement, but actually reversed it into a down-movement, shown by the up-curve (fig. 61).

The tabular statement on p. 119 explains the different results.

The diagrammatic representation, given in the four rows of fig. 62, offers further explanation of the responsive reaction, due to indirect stimulation of each quadrant by transmitted excitation from its corresponding sub-petiole being first neutralised and then reversed by direct stimulation of the opposite or antagonistic quadrant. The vertical series of figures to the left represent the transmitted excitation from sub-petiole (1), sub-petiole (4), sub-petiole (2), and sub-petiole (3), constituting indirect stimulation of the corresponding quadrants. The vertical series to the right, on the other hand, are symbolic of the simultaneous action of indirect stimulation from a sub-petiole and of direct stimulation of the opposite quadrant. The middle vertical row represents the effect of indirect stimulation I on one quadrant (dotted arrow), and of antagonistic effect of direct stimulation D (larger thick arrow) on the opposite quadrant. Detailed explanation of the series of diagrams in the middle of fig. 62 is given below.

First middle diagram.—Indirect stimulation I on quadrant (1), inducing moderate anti-clockwise torsion of that quadrant, is reversed by stronger effect of direct stimulation D of the antagonistic quadrant (4).

Second middle diagram.—Indirect stimulation I on quadrant (4), inducing moderate clockwise torsion, is reversed by stronger effect of direct stimulation D of the antagonistic quadrant (1).

Third middle diagram.—Indirect stimulation I on quadrant (2), inducing moderate rectilinear down-movement, is reversed by stronger effect of direct stimulation D of the antagonistic quadrant (3).

Fourth middle diagram.—Indirect stimulation I on quadrant (3), inducing moderate rectilinear up-movement, is reversed by stronger effect of direct stimulation D of the antagonistic quadrant (2).

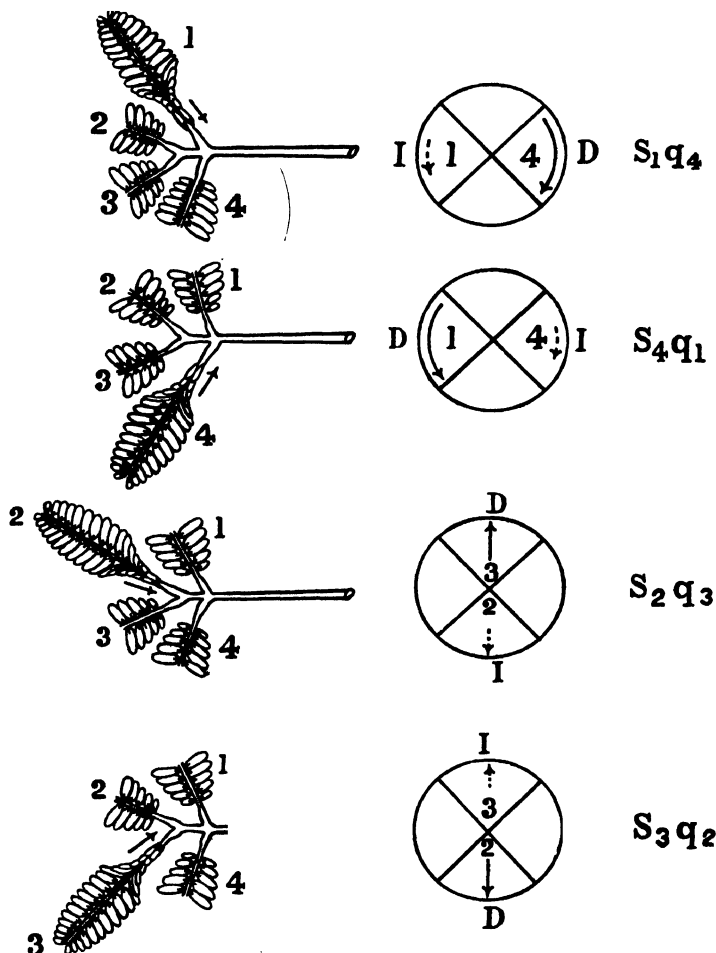


FIG. 62. Showing individual reactions and the resultant differential effect (*see text*).

All the above results are included in the statement that *the resultant effect is differential when the individual effect of indirect and direct stimulations are opposed to each other.*

Both the concordant and antagonistic effects are included in the general law that—

The resultant effect of indirect and direct stimulation is one of algebraic summation.

The highly complex results of the additive and differential effects of indirect and direct stimulation afford very conclusive proof of the existence of definite conducting tissue in the plant, analogous to the nervous tissue in the animal, the function of which is the transmission of excitation to a distance.

SUMMARY

The stimulation of each quadrant of the pulvinus of *Mimosa* can be effected directly ; it can also be effected indirectly by the transmitted excitation, due to stimulation of the corresponding sub-petiole situated at a distance. The left quadrant (1) is found to be in conducting communication with sub-petiole (1), the right quadrant (4) with sub-petiole (4), the lower quadrant (2) with sub-petiole (2), and the upper quadrant (3) with sub-petiole (3).

The above conclusion is established by experimental results that—

- i. The stimulation of each of the four sub-petioles at the periphery gives rise to an ingoing or centripetal impulse, which causes the characteristic response of the corresponding quadrant.
- ii. The effectual stimulation of the central end of the conducting tissue in each of the four quadrants gives rise to an outgoing or centrifugal impulse, which brings about the closure of the leaflets of the corresponding sub-petiole.

The characteristic reactions of the four quadrants of the pulvinus of *Mimosa* to indirect stimulation afford conclusive proof of definite conducting communication between the central and the peripheral ends of the petiole.

The effect of indirect stimulation at the periphery is accentuated by direct stimulation of the corresponding quadrant, an additive effect being thereby produced.

The effect of indirect stimulation at the periphery on any quadrant is, on the other hand, reversed by the direct stimulation of the antagonistic quadrant, the resultant effect being differential.

Each one of the numerous crucial tests employed offers independent and conclusive proof that the transmission of the impulse in the plant and in the animal is essentially of a similar nature, and is, in fact, the propagation of protoplasmic excitation.

VI.—THE DIRECTIVE ACTION OF AN ELECTRIC CURRENT ON TRANSMISSION OF EXCITATION IN *MIMOSA*

BY

B. K. DUTT, B.Sc.

A VERY important proof in support of the view that there is a similar mechanism for conduction of excitation in plants and animals is afforded by the modifying influence of the direction of a constant electric current which is maintained along the conducting tissue. It has been shown¹ that an electric current induces a parallel variation in the power of conduction of excitation in both plant and animal tissues, the characteristic effect depending on the direction and the intensity of the current.

The experiments on plants, referred to above, were carried out with *Mimosa pudica*, the petiole of which contains the conducting tissue. Local excitation was produced at the middle of the petiole by application of induction shocks of sub-maximal intensity. This gave rise to two excitatory impulses, one of which travelled outwards towards the periphery; the other impulse travelled inwards towards the centre, and on reaching the main pulvinus caused contraction of the motile organ, resulting in the fall of the leaf. The interval between the application of the stimulus and the initiation of the responsive fall of the leaf, as found from the automatic record given by the Resonant Recorder, enables the determination of normal velocity of transmission of excitation in the plant.

¹ Bose, 'The Influence of Homodromous and Heterodromous Current on Transmission of Excitation in Plants and Animals,' *Proc. Roy. Soc., B*, vol. 88 (1914).

The modifying influence of the action of a constant current was then investigated by maintaining an electric current of moderate intensity through the length of the petiole alternately in one direction or the opposite. The excitatory impulse, initiated at the middle of the petiole and transmitted inwards towards the pulvinus, could thus be made to travel either against the direction of the constant current or with that current. When the impulse is transmitted against the current, it is said to be travelling *up-hill*, and when in the same direction as the current it is described as travelling *down-hill*. The direction of the current may also be conveniently distinguished as *homodromous* when it flows in the same direction as the excitatory impulse, or *heterodromous* when the direction of the current is against that of the transmitted impulse. The effects of heterodromous and homodromous currents of moderate intensity on conductivity of the tissue were determined by measuring the variation induced in the velocity of transmission of excitation. The definite results obtained proved that the propagation of the impulse in the conducting tissue of the plant was enhanced by a heterodromous current, and retarded or completely blocked by a homodromous current. These effects were reversed when the intensity of the constant current was increased above a certain critical value.

Effects in every way similar were obtained with the nerve of the frog. Here also a heterodromous current of moderate intensity enhanced the conductivity of the nerve, while homodromous current inhibited or even blocked the power of conduction. Stronger intensity of the constant current caused, as in the case of plants, a reversal of the normal effects.

The demonstration of essential similarity of the conducting mechanism in plant and in animal being of great importance, I made attempts to extend the generalisation by employing the sub-petiole of *Mimosa* in the place of the petiole. The employment of the sub-petiole has the great advantage that the passage of the excitatory impulse along it is most vividly demonstrated by the successive closure of the attached leaflets. In the relatively easy method of

experiment to be presently described, it is unnecessary to employ elaborate instruments for determination of velocity of excitation, simple means being found for observing the course of the propagated impulse, its facilitation or its retardation, by the action of a heterodromous or a homodromous current.

METHOD OF EXPERIMENT

The very special advantage of the present method is that the opposite effects of the heterodromous and the homodromous current can be simultaneously demonstrated in each experiment, in which the excitatory impulse is generated by induction shocks applied near the central ends of a pair of sub-petioles. For this the terminals of an induction coil are connected by means of fine flexible wires to the inner ends of the sub-petioles. By the careful approach of the secondary coil towards the primary of the induction coil, the intensity of stimulus can be rendered either minimal or sub-maximal. Under sub-maximal stimulation the impulses in the two sub-petioles are simultaneously conducted to the peripheral ends, their propagation being strikingly manifested by the serial closure of the leaflets.

EXPERIMENTAL DEVICES

The illustration (fig. 63) shows the terminals of the secondary S of the induction coil being connected with the inner ends A and B of the pair of sub-petioles 1 and 4. The excitatory impulses initiated at A and B by the induction shock are propagated outwards from the base to the apex of the sub-petioles; the directions of transmission of the two impulses are represented by two arrows below the sub-petioles, the full arrow towards the right and the dotted arrow towards the left. These two impulses encounter heterodromous and homodromous currents, which are maintained by the passage of a constant electrical current represented in the illustration as entering the tip T' of sub-petiole 1 and leaving by the tip T of sub-petiole 4.

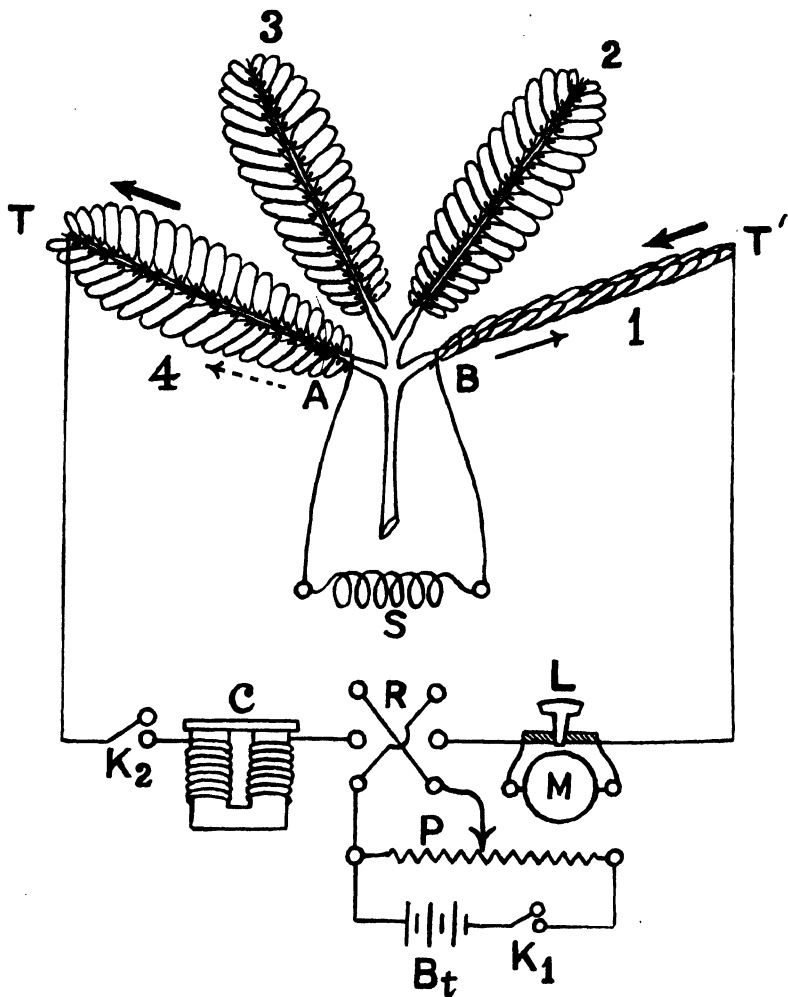


FIG. 63. Diagrammatic representation of the effects of homodromous and heterodromous currents of moderate intensity on transmission of excitation in the sub-petioles of *Mimosa*.

The testing shock from secondary coil S is applied at inner ends of sub-petioles 1 and 4; direction of propagated impulses shown by arrows below. On maintenance of constant current from T' to T, the impulse is facilitated against that of the constant current, and retarded against it. B_t, voltaic battery; P, potentiometer for adjustment of intensity of current; R, commutator; C, choking coil; M, micro-ammeter (see text).

The direction of the constant current is represented by thick arrows above the sub-petioles (fig. 63).

The illustration also explains how the excitatory impulse in the right sub-petiole has to travel *against* the direction of the constant current, which is therefore heterodromous. In the left sub-petiole the impulse has, on the other hand, to travel *with* the direction of the constant current, the latter being therefore homodromous. It will presently be shown that the direction of the constant current can be reversed by means of a commutator, when the impulse in the sub-petiole to the right encounters a homodromous current, that in the sub-petiole to the left a heterodromous current.

Special adjustments of the constant current circuit.—Means have to be found for changing the direction of the current as well as modifying its intensity. Steps have, moreover, to be taken for preventing the induction current employed for stimulation from entering the constant current circuit. The constant current is obtained from a battery of dry cells B_1 , which gives a maximum electromotive force of about 20 volts; the potentiometer P enables the exact adjustment of the derived E.M.F. for the constant current. The reversing key R is a Pohl's commutator by which the current can be sent from the right to the left, or in the opposite direction. The intensity of the current is measured by a micro-ammeter M , which is usually short-circuited by a plug key L . For measuring the current the key L is unplugged for a brief period.

The choking coil C is used for preventing the leakage of the alternating current from the induction coil into the circuit of the constant current. The choking coil has this special advantage that, while an alternating current is obstructed by it, a steady current can pass through it without hindrance.

Experiment 100. *Effect of homodromous and heterodromous currents of moderate intensity.*—The method of procedure is as follows: The application of induction shocks of sub-maximal intensity at the inner ends of the sub-petioles gives rise, as already explained, to two impulses which travel from the base to the apex, causing closure of all the

leaflets of the two sub-petioles in regular sequence. The intensity of the test stimulus employed is thus effective in bringing about conduction through the whole length of the sub-petioles. A period of about 20 minutes was next allowed for recovery and re-erection of the leaflets.

For observing the directive action of constant current on the propagation of excitation, the current was made to flow along the length of the two sub-petioles, in one of which it travelled from the periphery to the centre and in the other from the centre to the periphery. Special care has to be taken in regard to the manner of introducing the current, for a sudden make of the current causes excitation at the cathodic point of the sub-petiole. The possibility of this disturbance is removed if, instead of a sudden make of the current, it is *gradually* increased from zero to the maximum. This is secured by plugging the keys K_1 and K_2 and by gradually moving the rider of the potentiometer from the original zero to the maximum position. By suitably tilting the reversing key R, the current could be sent either from right to left or from left to right. The key is at first so arranged that the direction of the current in the sub-petioles is T' B A T. With this particular arrangement, as already explained, the current in the right sub-petiole is heterodromous, while in the left sub-petiole it is homodromous.

The induced variation of the conducting power of the tissue by the directive action of the constant current can be tested by applying as before the testing stimulus of sub-maximal intensity. This, it will be remembered, was effective in conducting excitation throughout the whole length of the right and the left sub-petioles. But during the passage of the constant current a marked variation in the conductivity of the two sub-petioles is induced. The homodromous current in the left sub-petiole is found to have blocked the passage of the impulse; this arrest of the conducting power is evidenced by the leaflets remaining undisturbed (*cf.* fig. 63).

The result is very different in the right sub-petiole in which the current is heterodromous. There is in this case

no obstruction to the passage of the impulse, which being effectively transmitted through the whole length of the sub-petiole, causes the serial closure of all its leaflets. Thus, in contrast to the action of the homodromous, the heterodromous current does not in any way obstruct the passage of the impulse; there is, on the other hand, reason to believe that it facilitates its passage (*cf.* Experiment 102).

Experiment 101. *Effect of reversing the direction of the current.*—In the last experiment the arrest of the excitatory impulse in the left sub-petiole occurred under the action of the homodromous current. That this is entirely due to the directive action of the current can be fully demonstrated by a corroborative reversal experiment repeated with the same specimen, the only difference being in the reversal of the current, which was now made to flow from T to T'.

In consequence of this reversal, the current in the left sub-petiole becomes heterodromous, while that in the right sub-petiole is homodromous. The observed results are very significant, fully corroborating the previous conclusions. The left sub-petiole, in which, in the previous experiment, the power of conduction had been arrested, now exhibited most effective transmission of the impulse, all its leaflets undergoing successive closure. The effect on the right sub-petiole is exactly the opposite to what occurred previously. The heterodromous current was then observed to have effectively transmitted the excitatory impulse, whereas the homodromous current now flowing in it effectively blocked the passage of the impulse, as evidenced by the fact that the leaflets of the sub-petiole remained completely unaffected. The results will be better understood from the statement in Table XIV.

The minimum current effective in inducing a marked variation of conductivity depends on the physiological condition of the specimen. At the beginning of winter, when the experiments were carried out, the effective intensity of the current varied from 1.4 to 2.8 micro-amperes. The electrical resistance offered by the length of the two sub-petioles was very considerable, and the effective E.M.F. was found to vary from 8 to 14 volts. The difference of

TABLE XIV.—EFFECT OF HOMODROMOUS AND HETERODROMOUS CURRENT OF MODERATE INTENSITY ON TRANSMISSION OF EXCITATION (SUB-PETIOLE OF *MIMOSA PUDICA*).

Experiment	Conducting organ	Direction of current	Effect induced
1. Current in one direction	Left sub-petiole	Homodromous	Arrest of conduction
	Right)	Heterodromous	Full conduction
2. Current reversed	Left sub-petiole	Heterodromous	Full conduction
	Right ,	Homodromous	Arrest of conduction

TABLE XV.—STATEMENT OF TYPICAL RESULTS OBTAINED WITH HOMODROMOUS AND HETERODROMOUS CURRENT OF MODERATE INTENSITY ON CONDUCTION OF EXCITATION.

Expt.	Testing Stimulus	Applied E.M.F.	Intensity of current	Effect of Homodromous current	Effect of Heterodromous current
1	0.3 unit	8 volts	2.8 micro-amps.	Complete block	Full transmission
2	0.3 "	8 "	1.4 "	"	"
3	0.3 "	10 "	1.4 "	"	"
4	0.4 "	10 "	2.8 "	"	"
5	0.4 "	14 "	2.8 "	"	"
6	0.3 "	14 "	1.4 "	"	"
7	0.3 "	12 "	2.8 "	"	"
8	0.4 "	14 "	2.8 "	"	"
9	0.3 "	14 "	2.1 "	"	"
10	0.4 "	14 "	2.8 "	"	"
11	0.3 "	14 "	2.1 "	"	"
12	0.3 "	14 "	2.8 "	"	"
13	0.4 "	14 "	2.1 "	"	"
14	0.3 "	12 "	1.4 "	"	"
15	0.4 "	14 "	2.1 "	"	"
16	0.4 "	12 "	2.1 "	"	"
17	0.3 "	12 "	2.1 "	"	"
18	0.4 "	12 "	2.8 "	"	"
19	0.4 "	10 "	2.1 "	"	"
20	0.3 "	12 "	2.1 "	"	"

conductivity induced by the constant current, homodromous or heterodromous, is best demonstrated by the employment of a testing stimulus which is somewhat below the sub-maximum intensity, *i.e.* about 0.3 to 0.4 unit of an arbitrary scale. In Table XV is given the typical results obtained with 20 different specimens.

Experiment 102. *Detection of variation of conduction under testing stimulus of minimal intensity.*—A very careful adjustment of the intensity of stimulus which is barely effective, is required for this investigation. This minimal stimulus causes transmission of excitation through only a short distance of the sub-petioles, as shown by the closure of a few leaflets near the point of application of stimulus. On sending the constant current a marked difference of reaction is noticed in the two sub-petioles. In the one in which the current is homodromous there is no closure of the leaflets, the conduction being blocked. In the other sub-petiole, in which the current is heterodromous, there is an enhancement of conductivity, as shown by the closure of the leaflets, not merely through a short length, but throughout the whole length of the sub-petiole.

EFFECT OF STRONG CURRENT

Experiment 103.—Under the action of a stronger current the normal effect exhibits a reversal. The homodromous current then enhances the power of conduction, while the heterodromous current causes an inhibition. The average

TABLE XVI.—EFFECT OF HETERODROMOUS AND HOMODROMOUS CURRENT OF STRONG INTENSITY.

Expt.	Testing stimulus	Applied E.M.F.	Intensity of current	Homodromous current	Heterodromous current
1	0.5 unit	24 volts	7 micro-amps.	Enhanced conduction	Block of conduction
2	0.5 "	26 "	7 "	"	"
3	0.6 "	24 "	7 "	"	"
4	0.4 "	26 "	6.3 "	"	"
5	0.7 "	24 "	5.6 "	"	"
6	0.7 "	24 "	7 "	"	"

intensity of current for bringing about a reversal is about 6.5 micro-amperes. The statement in Table XVI relates to results obtained with 6 different specimens.

SUMMARY

During the passage of a constant current a variation is induced in the conducting power for transmission of excitation in the plant. The variation induced depends on the direction and intensity of the current.

The power of conduction is enhanced by a current of moderate intensity when its direction is opposite to that of the propagated impulse, whereas it is enfeebled or blocked by a current which flows in the same direction as the impulse. In other words, a moderate heterodromous current enhances conductivity of the tissue for transmission of excitation, whereas a homodromous current depresses or arrests it.

It is also found that ineffectively transmitted excitation becomes effectively transmitted under a heterodromous current. Effectively transmitted excitation, on the other hand, becomes ineffectively transmitted or blocked under the action of a homodromous current.

The normal effect undergoes a reversal when the intensity of the current is above a critical intensity.

Parallel results, obtained with the nerve of a frog, prove that the conducting mechanism is essentially similar in the plant and in the animal.

VII.—ELECTRIC INVESTIGATIONS OF THE CONDUCTING CHANNELS IN THE LEAF OF *MIMOSA PUDICA*

BY

S. C. DAS, M.A.

THE pulvinus of *Mimosa pudica* is a complex organ, and may be regarded as consisting of four distinct quadrants—namely,

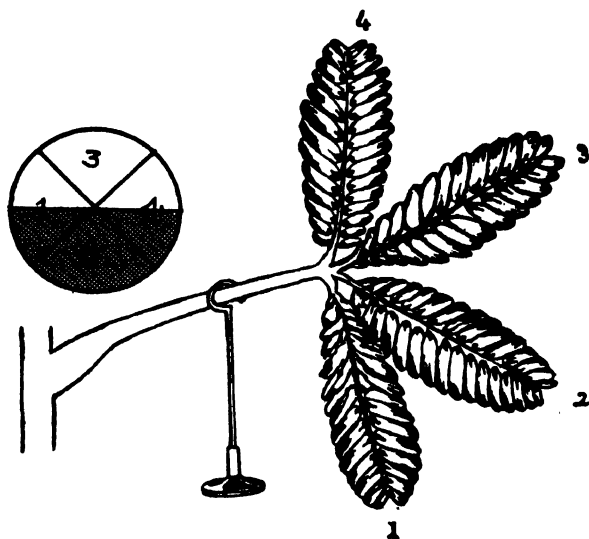


FIG. 64. The leaf of *Mimosa pudica* showing main pulvinus and four sub-petioles.

Diagrammatic representation of four quadrants of pulvinus seen in upper left corner. The quadrants are in conducting communication with corresponding sub-petioles.

the upper, the lower, and the two lateral ones. The different quadrants of the pulvinus are represented diagrammatically in the upper left corner of fig. 64. The quadrants to the

left and to the right are numbered (1) and (4) respectively, while the lower quadrant is numbered (2) and the upper quadrant (3). The sub-petioles are also so numbered that, to an observer standing in front of the stem, the successive sub-petioles, counting from the left, are in the sequence of (1), (2), (3), and (4). In the previous chapter the four quadrants have been proved to be in conducting communication with the corresponding four sub-petioles, similarly numbered.

It has been further shown that a characteristic mechanical response occurs at the central pulvinar end, by the centripetal impulse initiated at the periphery, which travels along a definite channel in the petiole. Thus stimulation of sub-petioles (1) and (4) induce respectively an anti-clockwise and a clockwise torsional movement of the leaf; stimulation of sub-petiole (2) gives rise to a vigorous rectilinear movement downwards; while that of sub-petiole (3) causes a less energetic rectilinear movement upwards. These characteristic responses of the four quadrants to indirect stimulation due to transmitted excitation from the four sub-petioles at the periphery, demonstrate the different conducting paths along which the excitatory impulse travels in the petiole.¹

The conducting communication between the centre and the periphery has also been independently demonstrated by the effect of centrifugal impulse, generated by the local stimulation of the central end of the conducting strand in each of the four quadrants of the pulvinus. Each impulse, travelling along a definite channel and arriving at the corresponding sub-petiole, causes the closure of its sensitive leaflets in proper sequence.

The present inquiry was undertaken with the view of demonstrating, by an altogether new method, the existence of specific conducting tissues which innervate the leaf of *Mimosa*. The method employed is that of electric response.

THE METHOD OF ELECTRIC RESPONSE

It is known that the state of excitation in an animal tissue can be detected by the induced electric variation, the

¹ Bose, *Nervous Mechanism of Plants* (1926).

excited point being galvanometrically negative in relation to the unexcited point. A similar effect in plants has also been demonstrated by special investigations carried out in the Bose Research Institute. The fact that the mechanical and electrical responses are but two different expressions of the same excitatory reaction has been proved by experiments with *Mimosa pudica*. The leaf of this plant was attached to an Optical Lever, which exhibited the mechanical response ; the responding organ was also suitably connected with a reflecting galvanometer for the exhibition of electric response. Stimulation was found to give rise not only to mechanical but also to electrical response of galvanometric negativity, both of which occurred practically at the same time. The special advantage of the electric method is that the excitatory electric reaction still occurs even when the motor organ is held rigid, preventing the manifestation of mechanical response.¹

ELECTRIC DETECTION OF CENTRIPETAL AND CENTRIFUGAL IMPULSES

The present series of investigations will be described in the following order :

- I. Electric detection of *centripetal* impulse in the leaf.
- II. Electric detection of *centrifugal* impulse in the leaf.

Both the centripetal and centrifugal impulses are generated by the action of stimulus. In demonstration of the universality of the phenomenon, it is necessary to employ not only one, but several independent methods of stimulation.

THE METHOD OF INVESTIGATION

The important conditions which it is necessary to secure are :

- i. Practical devices for different methods of stimulation, the intensity of which can be maintained constant or increased in a gradual manner.

¹ Bose, *Motor Mechanism of Plants* (1928), p. 146.

- ii. Suitable electric connections, either at the centre or at the periphery, for the detection of the arrival of the impulse initiated at a distance, the stimulation being *Indirect*.
- iii. A highly sensitive galvanometer for the record of the electromotive variation induced at the responding organ by the transmitted excitatory impulse.

A few words may here be said in regard to the minimum sensitivity of the recording galvanometer.

Sensitivity of the galvanometer.—The intensity of the electric current, due to induced electromotive variation, is very feeble, being of the order of 10^{-10} ampere; this should produce a deflection of 1 mm. on a scale placed at a distance of a metre. The electric response induced under indirect stimulation is recorded in the galvanograph on a moving photographic plate. An exceptionally sensitive galvanometer, which had been in constant use at the Institute for several years past, possessed a sensitivity as high as 10^{-11} , making it easy to secure a large amplitude in the recorded electric response. But after being in constant use for such a length of time its sensitiveness has undergone a decline, being at present reduced to about 10^{-9} . It is unfortunate that the galvanometers, generally available now, are not as sensitive as those supplied in pre-war days. Owing to the less sensitiveness of the galvanometer employed, satisfactory records of electric responses of *Mimosa* can only be obtained when the specimens are exceptionally vigorous. It should also be noted that the galvanometer used for the present records is not of a perfectly dead-beat type, and hence there is a tendency for the occurrence of a certain amount of after-oscillation.

Employment of Thermionic Amplifier.—An attempt was made to enhance the sensitivity for the purpose of record by the employment of thermionic valves. But this arrangement is subject to the great drawback that various external disturbances seriously affect the record. Again, the possibility of amplification by this method depends on the rapidity of the rate of electromotive variation. This rate is

not so rapid in the conducting tissue of the plant as it is in the conducting nerve of the animal. For these reasons it is doubtful whether this method of amplification of electric response of plants is likely to prove successful in securing perfectly reliable records.

Electric connection of plant with galvanometer.—The first contact is made with one of the responding quadrants of the pulvinus by thrusting a fine platinum wire into the tissue. The irritation caused by the slight wound disappears after a period of rest, when the normal excitability of the tissue becomes fully restored. The other contact is made at the distant indifferent area; for this the second platinum electrode is wrapped round the stem with a piece of cloth moistened in normal saline. The electric response is obtained photographically in a dark room by recording the excursion of a spot of light reflected from the mirror of the galvanometer. An important condition for obtaining satisfactory records is the maintenance of uniform sensitiveness of the *Mimosa* during the whole period of the experiment. For maintaining the normal excitability, the plant has to be kept in a place adjoining the photographic dark room, and exposed to light before a window; the plant otherwise becomes subtonic, with the result that its power of transmitting the excitatory impulse to a distance becomes arrested. An additional arrangement for securing constancy of light and temperature is the exposure of the plant to radiation from a 200 c.p. incandescent electric lamp.

Special precautions.—Great care has to be taken that wires from the pair of electrodes, which lead to the galvanometer in the photographic room, are perfectly insulated from the wall and floor of the experimental room. The base which supports the galvanometer has to be placed on a highly insulating stand and both kept free from any deposit of dust or moisture.

The amplitude of the recorded response depends on the intensity of transmitted excitation caused by indirect stimulation. Relatively weak stimulation induces a very feeble response; excessive stimulation, on the other hand, causes an overflow and a diffusion of excitation into the

neighbouring quadrants. If, however, a minimally effective stimulus be employed, then the induced galvanometric negativity is found to remain confined to the particular quadrant which is stimulated by transmitted excitation from the corresponding sub-petiole, the other quadrants remaining unaffected. This explains the necessity for careful adjustment of the minimally effective stimulus.

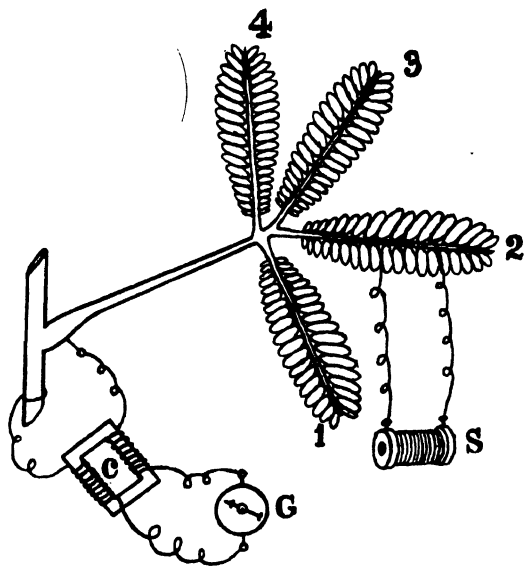


FIG. 65. Method for obtaining electric response of one of the four quadrants of pulvinus of *Mimosa*.

The sub-petiole (2) is stimulated by induction shock from secondary coil *s*, and the corresponding quadrant (2) is connected with galvanometer *G*. Choking coil *c* prevents leakage of shock-current into the galvanometer circuit.

The adjustment is effected without much difficulty by the employment of electric shocks from an induction coil, the intensity of which can be gradually increased by the approach of the secondary towards the primary coil. But the oscillatory induction shock is apt to introduce complications by the leakage of the shock-current into the galvanometer. This difficulty is, however, completely removed by the inclusion of a magnetic choking coil, which, while allowing

the induced E.M.F. to produce deflection of the galvanometer, nevertheless prevents the rapidly alternating current from entering the galvanometer circuit (fig. 65).

A different method for obviating the possibility of the passage of induction current into the galvanometer has also been perfected, in which the galvanometer is shunted just before the closure of the primary circuit of the choking coil, and remains shunted for a short time after the opening of the primary circuit.

ELECTRIC DETECTION OF CENTRIPETAL IMPULSE IN THE LEAF

Two different methods of indirect stimulation of leaf of *Mimosa* are employed in the following experiments—namely, the electrical and the radio-thermal.

ELECTRIC STIMULATION

Experiment 104. *Transmitted effect of electric stimulus of moderate intensity applied on sub-petiole (2).*—As a typical example of the general results, the effect of stimulation of sub-petiole (2) in inducing electric response of corresponding quadrant (2) of *Mimosa* is given in detail. A minimally effective induction shock of the intensity of 0.4 unit was found to transmit an impulse, which reached the particular quadrant after a short time and induced a responsive variation of galvanometric negativity of that quadrant, recorded as an up-curve. There was a recovery on the cessation of stimulation. It is to be noted that when the stimulus is of moderate intensity the response is single (fig. 66).

Experiment 105. *Transmitted effect of stronger stimulus.*—Keeping all other conditions the same as before, the sub-petiole (2) was next subjected to a stronger stimulus, the intensity being 1.0 unit; this resulted in transmission of stronger excitation and of an enhancement of the amplitude of response given by quadrant (2) which, as seen in fig. 67, is not single but multiple. Similar multiple response under strong stimulation is also observed in the record of mechanical

response of leaf of *Mimosa*. It is therefore very probable that the multiple electric response is a phenomenon parallel to the multiple mechanical response, and not essentially due to after-oscillation of the galvanometer.

Brief reference may here be made to the results already mentioned of the characteristic mechanical responses to excitation transmitted from each of the sub-petioles.

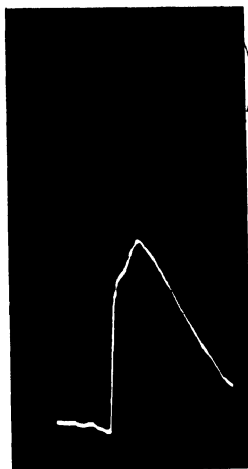


FIG. 66.

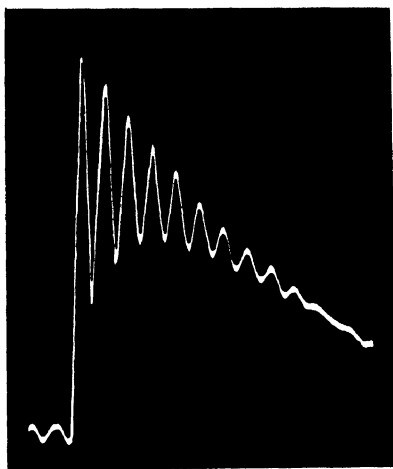


FIG. 67.

FIG. 66. Record of electromotive response of galvanometric negativity of quadrant (2) induced by transmitted excitation of indirect stimulus of moderate intensity from sub-petiole (2).

FIG. 67. Effect of stronger intensity of stimulus. Note large amplitude of response which is of multiple character.

Stimulation of quadrant (2) by transmitted excitation from sub-petiole (2) is found to give rise to an energetic rectilinear down-movement, while that of quadrant (3) by transmitted excitation from sub-petiole (3) shows less vigorous rectilinear up-movement. The responses of the quadrants (2) and (3) are thus antagonistic to each other, and can therefore be conveniently represented by records of opposite signs. Again stimulation of left quadrant (1) by transmitted excitation from sub-petiole (1) gives rise to an anti-clockwise

torsion, while that of right quadrant (4), stimulated by transmitted excitation from sub-petiole (4), exhibits a response of an opposite character—namely, that of a clockwise torsion.

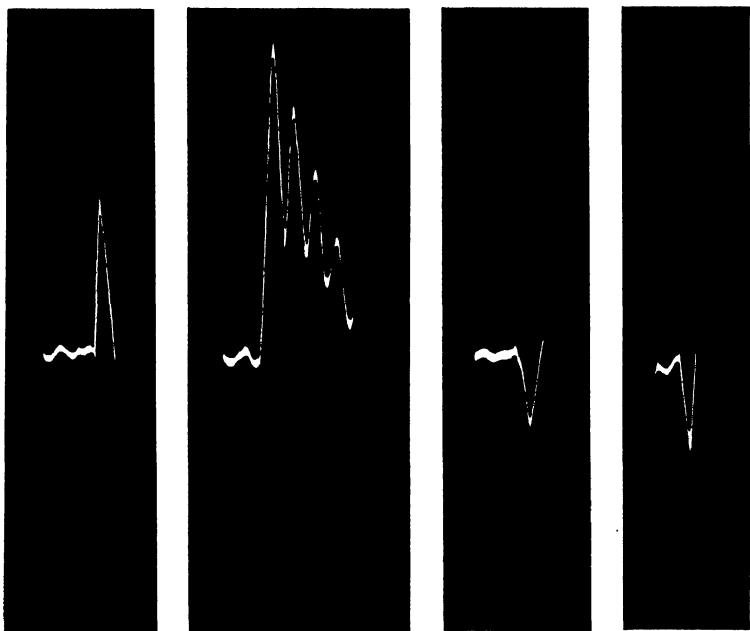


FIG. 68.

FIG. 69.

FIG. 70.

FIG. 71.

FIG. 68. Record of electromotive response of galvanometric negativity of quadrant (1) induced by excitation transmitted from sub-petiole (1).

In this and in the following records the different sub-petioles were subjected to electric stimulus.

FIG. 69. Response of quadrant (2) by transmitted excitation from sub-petiole (2).

Note multiple response.

FIG. 70. Electric response of quadrant (3) by transmitted excitation from sub-petiole (3).

FIG. 71. Electric response of quadrant (4) by transmitted excitation from sub-petiole (4).

The responses of the quadrants (1) and (4) are therefore also of opposite signs.

The electric responses of the antagonistic quadrants (2) and (3) will be distinguished in records of the following

experiments by up- and down-curves respectively. The responses of quadrants (1) and (4) will similarly be represented respectively by up- and down-curves.

After having described the typical result of excitation of the second quadrant by transmitted impulse from sub-petiole (2), the following results are given in proper sequence of the effects of indirect stimulation of sub-petioles (1), (2), (3), and (4).

Experiment 106. *Transmitted excitatory effect due to electric stimulation of sub-petiole (1).*—This gave rise to an excitatory response of galvanometric negativity of quadrant (1), represented by an up-curve (fig. 68).

The electric response of quadrant (2) to transmitted excitation from sub-petiole (2) has already been described in Experiment 105. A similar record obtained with a different specimen is given in fig. 69.

Experiment 107. *Transmitted excitatory effect due to electric stimulation of sub-petiole (3).*—This gave rise to response of galvanometric negativity of quadrant (3), represented by a down-curve (fig. 70), the amplitude being not so large as that of quadrant (2).

Experiment 108. *Transmitted excitatory effect due to electric stimulation of sub-petiole (4).*—This gave rise to electric response of galvanometric negativity of quadrant (4), represented by a down-curve (fig. 71).

In order to show that the effect is universal, the sub-petioles were next stimulated by a different mode of stimulation.

RADIO-THERMIC STIMULATION.

Radio-thermic stimulation is effected by means of a V-shaped platinum wire, rendered incandescent by the passage of an electric current from a storage battery. The duration of application is adjusted by means of a metronome. The different sub-petioles from (1) to (4) were in this way successively subjected to stimulation.

Experiment 109. *Transmitted excitatory effect due to radio-thermic stimulation of the different sub-petioles.*—The results

are precisely similar to those induced by transmitted excitation due to electric stimulation. Thus stimulation of

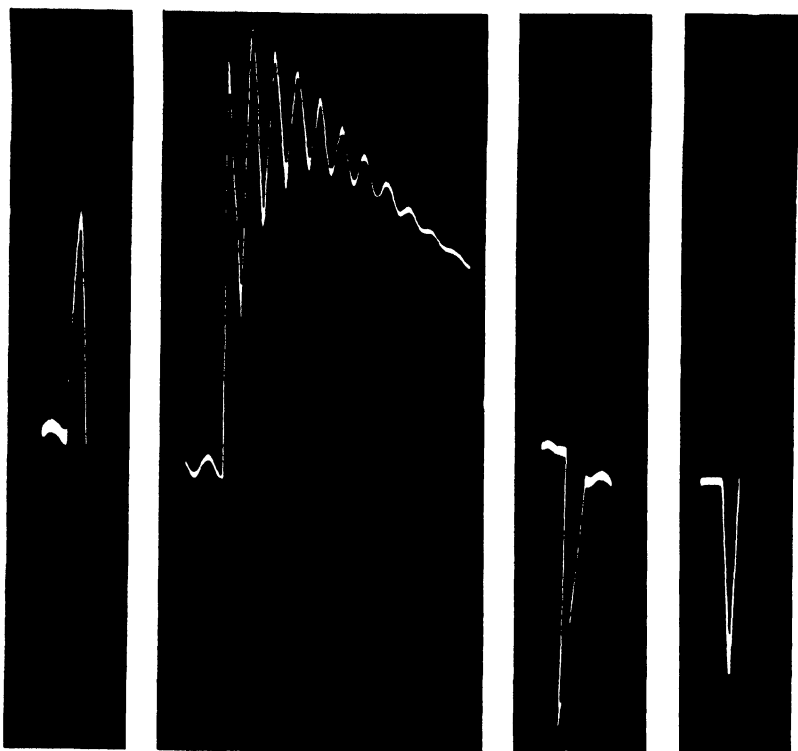


FIG. 72.

FIG. 73.

FIG. 74.

FIG. 75.

FIG. 72. Record of electromotive response of quadrant (1) due to transmitted excitation by stimulation of sub-petiole (1). In this and the following records the excitation of the different sub-petioles was effected by radio-thermal stimulus.

FIG. 73. Electromotive response of quadrant (2) due to excitation transmitted from sub-petiole (2).
Note multiple response.

FIG. 74. Electromotive response of quadrant (3) due to excitation transmitted from sub-petiole (3);

FIG. 75. Electromotive response of quadrant (4) due to excitation transmitted from sub-petiole (4).

sub-petiole (1) gave rise to galvanometric negativity of quadrant (1), exhibited as an up-curve (fig. 72). When the sub-

petiole (2) was stimulated the effect transmitted gave rise to an intense excitatory response of galvanometric negativity of quadrant (2), seen as a pronounced up-curve attended by multiple response (fig. 73). Stimulation of sub-petiole (3) gave rise to transmitted excitation which resulted in the electric response of galvanometric negativity of the corresponding quadrant (3), seen as a down-curve (fig. 74). Finally stimulation of sub-petiole (4) gave rise to excitatory response of galvanometric negativity of quadrant (4), shown as a down-curve (fig. 75). It will be noticed that the responses of quadrants (1) and (4) are of opposite signs, as are those of quadrants (2) and (3).

The experiments described above on electric detection of the centripetal impulse, travelling from the periphery to the centre, offer strong evidence of the special conducting path which connects each sub-petiole with its corresponding quadrant. This inference finds very strong additional support from the demonstration afforded by the electrical response of the secondary pulvinus, situated at the periphery, due to the centrifugal impulse initiated at the centre which now travels in a reverse direction, from the quadrants to the sub-petioles.

ELECTRIC DETECTION OF CENTRIFUGAL IMPULSE IN THE LEAF

In this investigation local stimulation is applied at the central ends of the conducting tissue in each of the four quadrants of the main pulvinus. Observation is then made of the electromotive variation, induced in the secondary pulvinus of the corresponding sub-petiole, by the excitatory impulse which is transmitted along definite channels from the centre to the periphery.

The stimulation at the centre was produced by the polar action of a constant current. It has been shown elsewhere that an excitatory reaction occurs locally at the cathodic point by the make of the electric current.¹ The special advantage in employing this method of electric stimulation

¹ Bose, *Motor Mechanism of Plants* (1928), p. 117.

is that, within limits, the excitation remains localised at the particular quadrant, and does not overflow into the neighbouring ones.

For obtaining record of electric response, one terminal of the galvanometer is connected with the secondary pul-

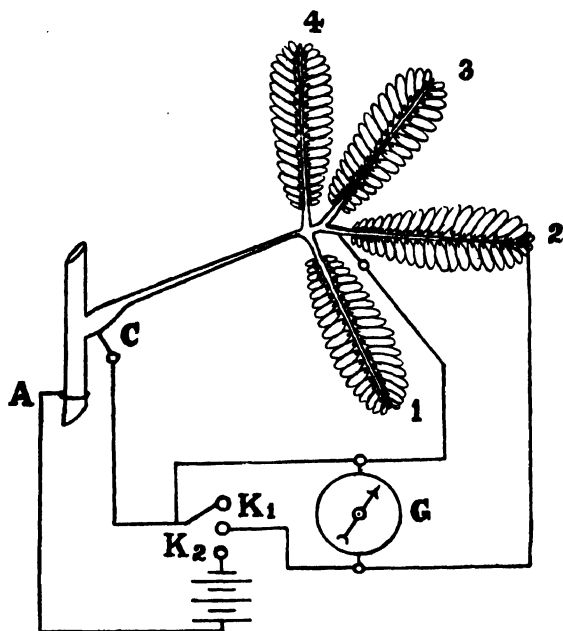


FIG. 76. Diagrammatic representation of the arrangement for local stimulation at the individual quadrant of pulvinus by constant current and the electric detection of transmitted excitation at the secondary pulvinus of the corresponding sub-petiole.

In this figure the lower quadrant (2) is made the kathode c, the anode being at indifferent point A on stem. Terminals of galvanometer G connected with secondary pulvinus and terminal leaflet of sub-petiole (2). The double press key K₁, K₂, effects the various operations with great rapidity.

vinus of the sub-petiole, while the other terminal is connected with an indifferent point at a distance—namely, with that of a single leaflet at the end of the same sub-petiole. This leaflet was previously killed by application of strong chloroform, so that its excitability was permanently abolished.

EXPERIMENTAL ARRANGEMENT

This is diagrammatically represented in fig. 76. For local stimulation of any quadrant, a fine sharp-pointed platinum wire is pricked into it so that, by proper manipulation of the key, it could be made a cathodic point C, anode A being at an indifferent point on the skin of the stem.

On completion of the polarising circuit by keys K_1 , K_2 , the excitatory impulse is initiated at the central end of the conducting tissue of the quadrant; the impulse thus generated reaches the secondary pulvinus after a short time and induces in it a responsive electromotive variation of galvanometric negativity. During the passage of this excitatory polarising current, the galvanometer is kept shunted for preventing any deflection caused by the leakage of the current into the galvanometer circuit. The polarising current is next stopped, the galvanometer being now unshunted for electric record of the effect of transmitted excitation at the secondary pulvinus. In practice the sequence of the completion of the polarising circuit for initiation of excitation, the shunting of the galvanometer, the breaking of the polarising circuit, and the unshunting of the galvanometer, are effected with great rapidity by means of a double press key which is diagrammatically represented in the illustration.

TRANSMITTED EFFECT OF POLAR EXCITATION BY
CONSTANT CURRENT

Experiment 110. *Effect of transmission of centrifugal impulse initiated at quadrant (1).*—This gave rise to an electric response of galvanometric negativity of the secondary pulvinus of sub-petiole (1), recorded as an up-curve (fig. 77).

Experiment 111. *Effect of transmission of centrifugal impulse initiated at quadrant (2).*—The result of this was the response of galvanometric negativity induced in the secondary pulvinus of sub-petiole (2), shown as an up-curve (fig. 78).

Experiment 112. *Effect of transmission of centrifugal*

impulse initiated at quadrant (3).—The excitatory reaction of the transmitted impulse was the induced galvanometric negativity of the secondary pulvinus of sub-petiole (3), shown as a down-curve (fig. 78).

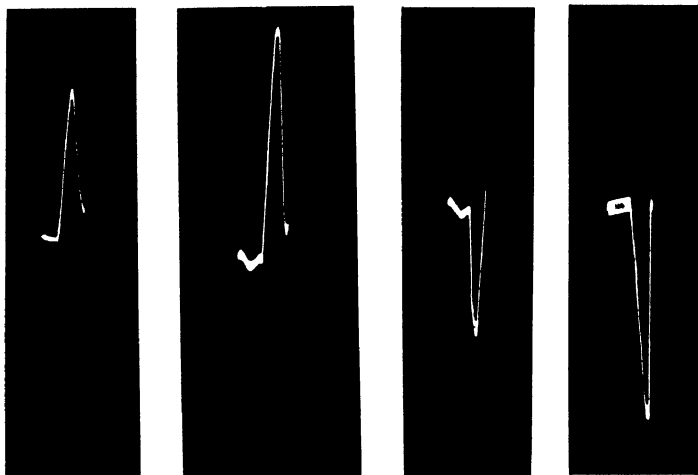


FIG. 77.

FIG. 78.

FIG. 79.

FIG. 80.

FIG. 77. Response of galvanometric negativity induced in secondary pulvinus of sub-petiole (1) by transmitted excitation from quadrant (1).

In this and in the following records, stimulation at the central end of the conducting tissue was effected by the polar action of a constant current.

FIG. 78. Electric response of galvanometric negativity induced in secondary pulvinus of sub-petiole (2) by transmitted excitation from quadrant (2).

FIG. 79. Electric response of galvanometric negativity induced in secondary pulvinus of sub-petiole (3) by transmitted excitation from quadrant (3).

FIG. 80. Electric response of galvanometric negativity induced in secondary pulvinus of sub-petiole (4) by transmitted excitation from quadrant (4).

Experiment 113. *Effect of transmission of centrifugal impulse initiated at quadrant (4).*—This gave rise to an electric response of galvanometric negativity of secondary pulvinus of sub-petiole (4), recorded as a down-curve (fig. 80).

SUMMARY

In addition to the results described in previous chapters, the existence of special impulse-conducting strands in the petiole of *Mimosa* is independently demonstrated by the electric response to centripetal impulse from the periphery to the centre, and to centrifugal impulse from the centre to the periphery.

These impulses are generated, not by any one mode of stimulation, but by diverse modes, such as induction shocks, radio-thermal stimulus, and polar excitation by constant current.

The *centripetal* impulse initiated at a particular sub-petiole is found to be transmitted in an ingoing direction from the periphery to the centre along a definite channel, and to induce electromotive variation of galvanometric negativity of the corresponding quadrant of the pulvinus.

The *centrifugal* impulse, generated at the central end of the conducting tissue of a quadrant is, on the other hand, propagated in an outgoing direction from the centre to the periphery along a definite channel, giving rise to the characteristic electric response of galvanometric negativity of the secondary pulvinus of the corresponding sub-petiole.

The results of these experiments afford convincing proof of the existence of definite conducting tissues constituting the innervation of plants, the function of which is analogous to the nervous tissues in the animal.

VIII.—MODIFYING EFFECT OF CHANGE OF ENVIRONMENT ON THE IRRITABILITY OF *NEPTUNIA OLERACEA*

BY

S. C. DAS, M.A.

NEPTUNIA oleracea is an aquatic plant growing wild in ponds and streams in Bengal and in neighbouring provinces. Its normal habitat, however, can be changed from water into land by means of cuttings. When growing in water the stem of the plant is partially submerged, being kept afloat by means of a corky tissue, the leaves thus remaining above water. *Neptunia* can also be grown on land by planting cuttings in moist earth or by direct germination of the seed in moist soil. The plants become adapted to the new environment in the course of a month, when their stems and leaves appear to be changed, becoming somewhat thin.

The subject of the present inquiry is the experimental determination of the modification of the inner activities of the plant, induced by the change of habitat from water to land. For this, investigations were undertaken on modification of various aspects of irritability of the plant, and attempts made to obtain quantitative results. The measurements related to :

- (1) The contractility of the pulvinus.
- (2) The characteristics of recovery from excitation.
- (3) The apex time or period of maximum contraction.
- (4) The latent period.
- (5) The conduction of excitatory impulse.

MEASUREMENT OF CONTRACTILITY OF PULVINUS

The contractility of pulvinus is determined by the amplitude of the responsive movement induced under a given stimulus. The difference in the power of contraction is likely to be exhibited in two different ways. The more active pulvinus, whether in water or in land specimens, would show

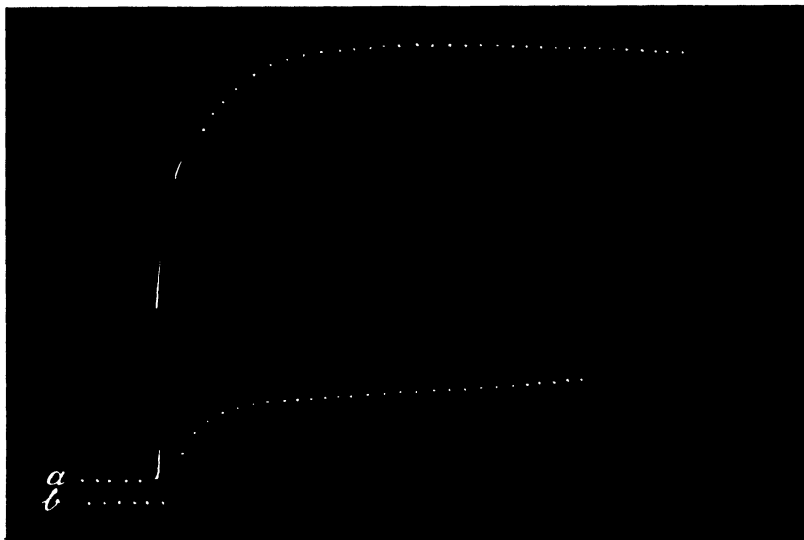


FIG. 81. Record of contractile response.

- (a) Upper record represents the response-curve of leaf of land-grown *Neptunia*.
 (b) Lower record is that of aquatic *Neptunia*.

Dots at intervals of 4 seconds; owing to rapid rate of contractile movement of the land specimen, the dots appear as dashes.

a quicker rate of responsive movement; the amplitude of its curve of response should also prove to be larger.

Experiment 114. *Determination of the contractility of the pulvinus.*—In order to study the characteristics of the curve of response of the two classes of specimens, grown under such widely different conditions as water and land, suitable specimens cut from parent plants were mounted in U-tubes, with the cut end immersed in water. These specimens

were next enclosed within a moist chamber, and the recovery from shock due to cut was found to be completed after a period of rest of two hours. The pulvinus was then subjected to direct stimulation by induction shocks of intensity of 2 units, the duration of application being maintained constant in successive experiments by the use of a metronome. The curves of response were obtained with the Oscillating Recorder on a moving smoked-glass plate, the time-interval between successive dots, unless stated to the contrary, being 4 seconds. The curves of response of the land and of the water specimens were obtained under identical conditions of temperature and humidity. The magnification produced by the writing lever in this particular experiment was 6 times.

The upper curve (fig. 81) is the record of the response of the leaf of land-grown *Neptunia*, and the lower curve that of the plant grown in water. A glance at the two curves, one below the other, at once reveals the far greater contractility of the land-grown *Neptunia*. The rate of its responsive movement, seen in the first portion of the curve, is so rapid that the dots are prolonged into dashes. The vertical spacings, moreover, are very much wider in the upper curve, as compared with their relative closeness in the lower, indicating the greater contractile activity of the land-grown specimen. The amplitude of response, as shown in the upper curve, is 60 mm., whereas it is only 15 mm. in the lower. The contractility of the land-grown *Neptunia* is thus four times greater than that of the specimen grown in water.

In Tables XVII and XVIII are given measurements of contractility of four other typical specimens of the two classes, in which the records were taken somewhat differently. As the contractile response of the water specimen is very feeble, the magnification employed for its record was, in these cases, 16 times, while that of the more active land specimen was only six times. The true amplitude of response in millimetres is obtained by dividing the amplitude of the recorded response by the magnifying power.

The ratio of the contractile powers of land and water specimens of *Neptunia* is found from the Tables as

12·6 : 2·75. The contractility of the pulvinus of the land-grown *Neptunia* is thus 4·5 times greater.

TABLE XVII.—MOTO-EXCITABILITY OF *NEPTUNIA* GROWN ON LAND.

(Magnification of record 6 times.)

Specimen	Magnified amplitude of response	True amplitude of response
1	63 mm.	10·5 mm.
2	64 „	10·7 „
3	80 „	13·3 „
4	96 „	16·0 „
Mean value = 12·6 mm.		

TABLE XVIII.—MOTO-EXCITABILITY OF *NEPTUNIA* GROWN IN WATER.

(Magnification of record 16 times.)

Specimen	Magnified excitability of response	True amplitude of response
1	35 mm.	2·2 mm.
2	40 „	2·5 „
3	49·5 „	3·1 „
4	51 „	3·2 „
Mean value = 2·75 mm.		

THE CHARACTERISTICS OF RECOVERY

Experiment 115. *Determination of period of recovery.*—The pulvinus of the plant is the motor organ which undergoes contraction under stimulation. After the attainment of maximum contraction of the pulvinus, recovery sets in.

Two curves were obtained, one of land-grown *Neptunia*, and the other of the water-grown type. The general experimental arrangement was the same as in the previous experiment. Records were taken with the Oscillating Recorder, the successive dots being now at intervals of 30 seconds. For facility of comparison, the magnification employed for record of feeble contractile water-grown

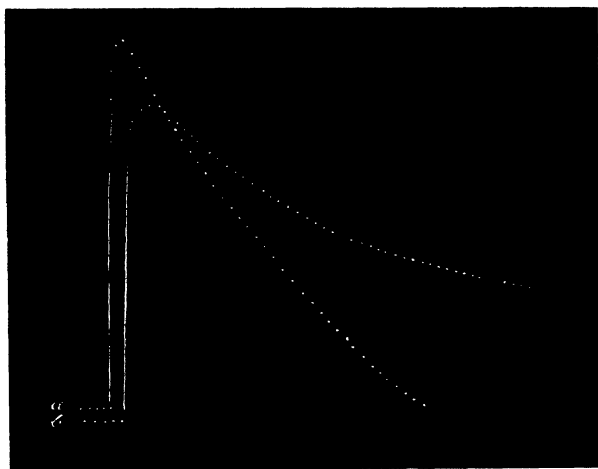


FIG. 82. The curve of response-recovery of *Neptunia*.

- (a) Upper record given by land specimen exhibits quicker rate of recovery.
- (b) Lower record of aquatic specimen shows extremely slow rate of recovery.

It is to be noted that the curve of recovery of the upper record crosses that of the lower.

Neptunia was 4 times greater than that for the land specimen. The curve of recovery in the water-grown variety is much more flat, as seen in the lower record (fig. 82), compared with the more erect response of the land-grown specimen, seen in the upper record, the second portion of which crosses the lower. Complete recovery was attained in the course of 17 minutes in a land specimen, whereas in the aquatic variety the recovery was only partial even after 30 minutes.

THE APEX TIME

Experiment 116. *Determination of the apex time.*—By the term Apex Time is meant the time-interval between the initiation of response and the attainment of the maximum contraction of the pulvinus of the leaf. The upper record (fig. 83) gives the apex time of *Neptunia* grown on land, while the lower record gives that of an aquatic specimen.

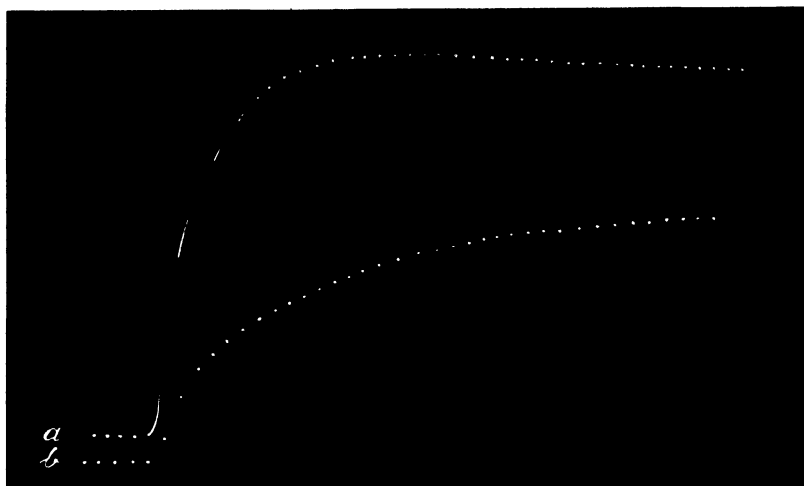


FIG. 83. Record of apex time of *Neptunia*.

(a) Upper record from land specimen.

(b) Lower curve from aquatic specimen.

Note shorter apex time for land-grown *Neptunia*. Dot-intervals 4 seconds.

The characteristics of the two curves clearly demonstrate the far quicker reaction in a land specimen, as exhibited by the erectile nature of the curve, in marked contrast with the very sluggish reaction shown by the flat curve in a water-grown variety. In the particular records the apex time is 36 seconds in the land specimen, and 128 seconds in the aquatic specimen; the maximum contraction is thus effected 3.5 times quicker in the land specimen than in the aquatic *Neptunia*.

The two following tables give the relative values of apex time in six typical specimens of each kind.

TABLE XIX.—GIVING THE APEX TIME IN LAND-GROWN *NEPTUNIA*.

Specimen	Apex time in seconds
1	28 seconds
2	36 "
3	36 "
4	36 "
5	40 "
6	48 "
Mean value = 37 seconds	

TABLE XX.—GIVING THE APEX TIME IN AQUATIC *NEPTUNIA*.

Specimen	Apex time in seconds
1	80 seconds
2	104 "
3	104 "
4	108 "
5	124 "
6	128 "
Mean value = 108 seconds	

The contractile reaction is thus far more sluggish in aquatic specimens, the apex time of which is about three times longer.

THE LATENT PERIOD

When a motile organ is directly stimulated by an induction shock, a short period elapses between the moment of application of stimulus and the initiation of the responsive

movement. This brief interval is designated as the Latent Period.

Experiment 117. *Determination of the latent period of pulvinus of Neptunia.*—The record was obtained by means of the Resonant Recorder, the frequency of vibration of the tapping writer being ten times per second. The experimental method consists in connecting one electrode of the induction coil with the petiole, the other electrode being connected with the stem. The pulvinus, interposed in the path of the induction shock, was thus directly stimulated, the intensity of the shock being 2 units.

TABLE XXI.—GIVING THE LATENT PERIOD IN *NEPTUNIA* GROWN ON LAND.

Specimen	Latent period in seconds
1	0.40 second
2	0.45 „
3	0.45 „
4	0.45 „
5	0.45 „
6	0.50 „
Mean value = 0.45 second	

TABLE XXII.—GIVING THE LATENT PERIOD IN AQUATIC *NEPTUNIA*.

Specimen	Latent period in seconds
1	1.2 second
2	1.3 „
3	1.5 „
4	1.6 „
5	1.7 „
6	2.1 „
Mean value = 1.6 second	

In a pair of typical experiments with the two kinds of *Neptunia*, the latent period of the pulvinus of the specimen grown on land was found to be 0.4 second. In contrast with this was the very much longer latent period of pulvinus of the aquatic specimen, which was 1.3 second.

Tables XXI and XXII give the latent period of pulvinus of *Neptunia* in six typical cases of each kind.

The average value of latent period of aquatic *Neptunia* is thus about 3.5 times longer than that of land specimens.

THE CONDUCTION OF EXCITATORY IMPULSE

Experiment 118. *Determination of the velocity of transmission of excitation.*—The power of conduction of excitation of *Neptunia* was determined by the Electro-magnetic Tapping Recorder. The successive dots were adjusted to be exactly at intervals of one second. An intensity of induction shock of 2 units was applied at a point on the petiole at a distance of 10 mm. from the pulvinus. The total time-interval T between the moment of application of stimulus and initiation of response was determined from the record. Subtracting from this the average value of the latent period of the pulvinus, the actual or true time of transmission t of the impulse through the intervening distance d of the petiole is found by subtracting the latent period L from the observed time of transmission T ; that is to say, $t = T - L$.

The velocity of transmission V is measured by the formula $V = \frac{d}{t} = \frac{d}{T - L}$

The conductivity of the petiole of *Neptunia oleracea* grown in water was so feeble that a stimulus applied on the petiole at a distance of 10 mm. from the pulvinus hardly evoked any perceptible movement of response at the distant pulvinus. The petiole of aquatic *Neptunia* may therefore be regarded as practically non-conducting when the stimulus is not of abnormally high intensity. The petiole of specimen

grown on land was, however, found to be moderately conducting.

In the record (fig. 84) the point of application of stimulus on the petiole was at a distance of 10 mm. from the pulvinus. The total time of transmission was 5 seconds, and the average value of the latent period, as determined by the Resonant

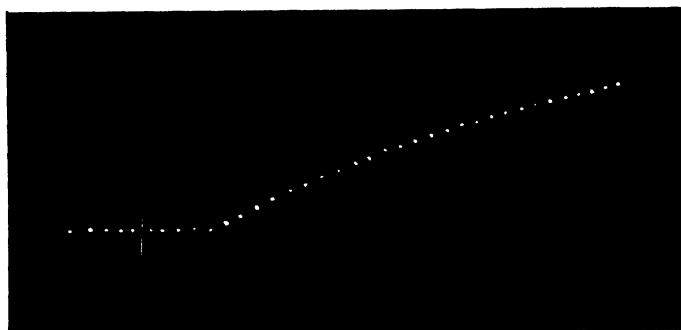


FIG. 84. Determination of velocity of transmission of excitation in *Neptunia*.

Record shows the time-interval for transmission through 10 mm. length of petiole of specimen grown on land.
Dots at intervals of 1 second.

Recorder, was 0.45 second (*cf.* Table XX). The velocity of transmission of excitation in the petiole of land-grown *Neptunia* is thus :

$$V = \frac{10}{5 - 0.45} = 2.2 \text{ mm. per second.}$$

Under favourable circumstances, such as season and high temperature, the velocity of transmission was found to be appropriately increased. This velocity of transmission of excitation in the petiole of *Neptunia* is of the same order as that in the stem of *Mimosa pudica* ; but the velocity in the petiole of *Mimosa* is considerably higher, being about 26 mm. per second.

The following table gives the velocity of transmission of excitation in the petiole of three average specimens of *Neptunia* grown on land :

TABLE XXIII.—VELOCITY OF TRANSMISSION OF EXCITATION IN PETIOLE OF LAND-GROWN *NEPTUNIA*.

Specimen	Distance d of trans- mission	Total time T	Latent period L	Conductivity $V = \frac{d}{T-L}$
1	10 mm.	5 sec.	0.45 sec.	2.2 mm. per sec.
2	10 "	5 "	0.45 "	2.2 " " "
3	6 "	3 "	0.45 "	2.3 " " "
Average velocity of transmission = 2.2 mm. per second				

It would be interesting to compare various aspects of irritability of *Mimosa pudica* with those of *Neptunia* grown on land and in water. The following table gives the characteristic values in different specimens:

TABLE XXIV.—DIFFERENT ASPECTS OF IRRITABILITY IN *MIMOSA PUDICA* AND IN LAND AND WATER *NEPTUNIA*.

Specimen	Apex time in seconds	Latent period in seconds	Rate of conduction in mm. per second
<i>Mimosa pudica</i> .	1.25 sec.	0.1 sec.	26 mm. per sec.
Land <i>Neptunia</i> .	37.0 "	0.45 "	2.1 " " "
Aquatic <i>Neptunia</i>	108.0 "	1.6 "	Negligible

SUMMARY

The environment and habitat play a very important part in modifying the life activity of an organism. This is exemplified by the variation induced in the internal activities of *Neptunia* by the change of its habitat from water into land.

The invisible internal change that has been induced in the plant can be revealed by the application of a testing

stimulus, and by recording the characteristic variation of different aspects of its irritability.

The average contractility of the pulvinus of *Neptunia*, grown in land, is found to be more than four times that of the aquatic plant. The period of its recovery from stimulation is also much quicker.

The average apex time of the pulvinus of land-grown *Neptunia* is about three times quicker than that of the aquatic specimen. The latent period of pulvinus of land-grown *Neptunia* is about 3.5 times quicker than that of the aquatic kind.

The power of conduction of excitation to a distance is exceedingly feeble or even absent in aquatic *Neptunia*; but in the land-grown specimen the velocity of conduction is about 2 mm. per second.

IX.—ANISOTROPY INDUCED IN PLANTS UNDER UNILATERAL STIMULATION BY LIGHT AND BY GRAVITY

BY

U. C. SEN, M.Sc.

THE main pulvinus of *Mimosa* may be regarded as a naturally anisotropic organ, since its upper and lower halves are unequally excitable. When a localised stimulus of the same intensity is alternately applied on the upper and lower halves, the amount of responsive movements is very different ; thus while stimulation of the upper half induces a slight contraction of that half, indicated by a relatively feeble up-movement, similar stimulation of the lower half induces a very marked contraction of that half, exhibited by a more energetic and intense down-movement. On account of this differential excitability a diffuse stimulation, say by an electric shock, acting simultaneously on both the upper and the lower halves, gives rise to a resultant down-movement, by the predominant contraction of the more excitable lower half.

The direction of resulting movement, caused by diffuse stimulation, thus reveals the more excitable half of the organ, as that particular half undergoes greater contraction.

The excitatory reaction in plant tissue can also be detected by means of electric response. It has been shown that all plants and all their organs are excitable, the state of excitation being detected by concomitant change of galvanometric negativity.¹ The results are more or less quantitative, for while stimulation of a feebly excitable tissue gives rise to a feeble negative electromotive varia-

¹ Bose, *Response in the Living and Non-living* (1902).

tion, a highly excitable tissue exhibits, under parallel conditions, a strong electromotive variation of galvanometric negativity.

It has also been shown that an anisotropic organ, under diffuse stimulation, exhibits not only greater contraction but also greater galvanometric negativity of its more excitable side. From the results thus obtained the following laws were discovered and formulated as follows¹:

LAW I. ON SIMULTANEOUS EXCITATION OF TWO POINTS A AND B, THE RESPONSIVE CURRENT FLOWS IN THE TISSUE FROM THE MORE TO THE LESS EXCITED POINT.

LAW II. CONVERSELY, IF UNDER SIMULTANEOUS STIMULATION THE RESPONSIVE CURRENT BE FROM B TO A, THEN THE POINT B IS RELATIVELY THE MORE EXCITABLE.

The anisotropy of an organ can thus be detected by means of its electric response. All that is necessary is to connect the terminals of a sensitive galvanometer with the two points A and B, and subject the organ to diffuse stimulation; if there is no resulting current of response, then the organ must be isotropic, its different sides being equally excitable; in such a case the induced galvanometric negativity of A and B being equal, there is no resultant current. But if the induced galvanometric negativity of the two points are unequal then the organ must be anisotropic; *a greater negativity of, say, B, leading to the flow of the responsive current from B to A, would then prove B to be the more excitable.*

In some plants the anisotropy of the organ is natural, as in the primary pulvinus of *Mimosa*. In other cases the anisotropy may be induced by the differential action of stimulus on different sides of the organ.

In the following investigations, experiments will be described on the electric response of naturally anisotropic organs, as well as of those in which the anisotropy has been induced by the differential action of stimulus.

¹ Bose, *Comparative Electro-physiology* (1907).

ELECTRIC RESPONSE OF NATURALLY ANISOTROPIC
ORGANS

Experiment 119. *The response of pulvinus of Mimosa.*—The terminals of a highly sensitive galvanometer were connected with the upper and lower halves of the pulvinus, and the electric response of the organ recorded under diffuse stimulation. One of the means of diffuse stimulation of all sides of the organ is by shocks from an induction coil. The complication arising from the leakage of the shock-current is avoided by the interposition of a magnetic choking-coil, which prevents the rapidly alternating current from entering the galvanometer circuit (*cf.* fig. 65). In the present experiment an induction shock of moderate intensity was applied on the petiole of the leaf at a short distance from the pulvinus; the impulse that was initiated reached the pulvinus and induced simultaneous excitation of both the upper and lower halves of the organ. The direction of the resulting current of electric response was across the pulvinus from the lower to the upper half, the lower being thus proved to be relatively the more excitable (fig. 85).

The electrical response is independent of the mechanical, for it occurs even when the leaf is held rigid, thus preventing any movement. From this it follows that in an organ which does not exhibit any marked motility the differential excitability can still be determined by means of electric response.

Natural Anisotropy of Tendrils.—In a tendril two opposite sides can be distinguished, one of which is slightly concave and the other convex. Mechanical friction of the concave side induces a contractile response by a curling movement, whereas similar stimulation of the convex side is without any marked effect. The tendril is thus seen to be an anisotropic organ, its concave side being the more excitable.

Experiment 120. *Electric response of anisotropic tendril of Vitis quadrangularis.*—For obtaining electric response the galvanometric connections were made with two diametrically opposite sides of the tendril, one on the convex and the other on the concave side. After this, simultaneous

excitation of the opposite sides of the organ by induction shocks was found to give rise to a definite directioned electric response, the current flowing across the tendril from the concave to the convex side. This demonstrated that

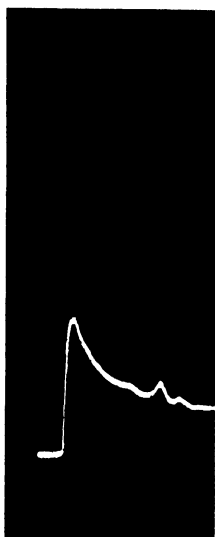


FIG. 85.

FIG. 85. Electric response of the pulvinus of *Mimosa pudica* under diffuse stimulation.

The direction of responsive current across the pulvinus is from the lower to the upper half, proving the greater excitability of the lower half.



FIG. 86.

FIG. 86. Two successive responses of tendril of *Vitis quadrangularis*.

The current of response is from the concave to the convex side, the concave being thus proved to be the more excitable.

the concave is the more excitable side of the organ. Two successive electric responses obtained with the tendril are reproduced in fig. 86.

ANISOTROPY INDUCED BY DIFFERENTIAL STIMULATION

In the cases described above the anisotropy was natural. The question arises whether anisotropy can be induced in an

isotropic organ by the differential action of stimulus on its two opposite sides. In solving this problem the electric method of detection of anisotropy will be used ; for diffuse stimulation of the organ various modes of testing stimulus will be employed. These diverse modes are :

- (1) Stimulus of Induction Shock,
- (2) Stimulus of Torsional Vibration, and
- (3) Stimulus of Transverse Section.

Stimulation by induction shock has already been described. As regards mechanical stimulation, rapid torsional vibration is found to be a very effective means for simultaneous stimulation of different sides of the organ. A transverse cut acts also as an intense stimulus, the effect of which does not remain localised, but is conducted to the neighbouring tissue, the distance of transmission being dependent on the conductivity of the organ.

INDUCED ANISOTROPY DUE TO DIFFERENTIAL STIMULATION BY LIGHT

A radial erect stem maintained in darkness is an isotropic organ, its different sides being equally excitable ; this characteristic also persists when the vertical stem is equally illuminated on all sides by light from above. It is obvious that, in both cases, all sides being subjected to similar conditions, no differential excitability can be induced. But in a procumbent as well as in a creeping stem, as previously explained by Sir J. C. Bose, there is a differential stimulation on the upper and the lower sides. He has shown that when the upper side is constantly exposed to light, over-stimulation induces fatigue and diminution of excitability of that side. Thus under the differential effect of light an originally isotropic organ is made anisotropic, the upper exposed side being rendered relatively the less excitable. The effect of unequal action of light is, in such cases, further accentuated by the differential action of geotropic stimulus. The electric investigation on the subject was carried out by Sir J. C. Bose on procumbent stems of *Cucurbita*.

Following similar methods I experimented with diverse species of plants with the object of detecting the induction of anisotropy of an organ under the action of unilateral light. The following experiments give the typical results.

Experiment 121. *Electric response of procumbent branch of Impatiens*.—A short length of a horizontal branch of *Impatiens* was mounted in the Torsional Vibrator. In this apparatus there are two clamps, A and B, holding the

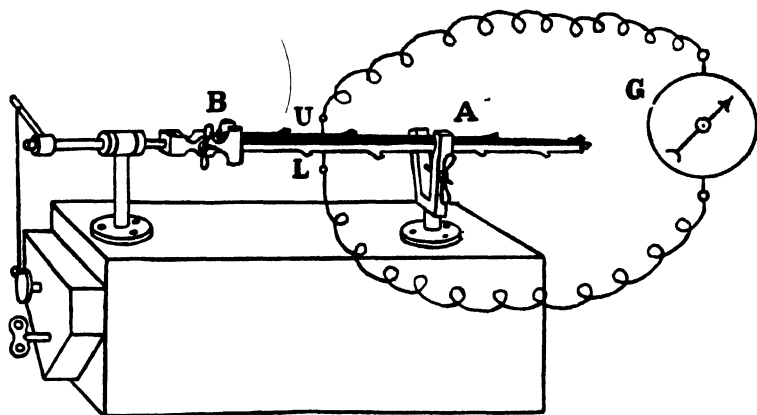


FIG. 87. Apparatus for stimulation by Torsional Vibration. A, clamp for holding plant in fixed position. B, clamp for holding free end of plant for subjecting it to torsional vibration by rapid alternating rotation in clockwise and anti-clockwise directions.

The upper or exposed side is shown as dark.

specimen in such a way that the darker sun-exposed side U is seen in the illustration as the upper, L being the lower unexposed side. Of the two clamps A is stationary, while B can be rapidly rotated by clockwork in alternate clockwise and anti-clockwise directions, thus causing diffuse mechanical stimulation (fig. 87). The intensity of this testing stimulus can be suitably modified by variation of the angle of rotation and the duration of vibration.

For electric connections with the galvanometer, two thin platinum electrodes, soldered to tinsel, are pricked into the upper and lower sides of the organ. The pieces of tinsel are led to the terminals of a sensitive galvanometer. The

flexibility of the tinsel prevents relative movement of the plant and the electrodes during the torsional vibration.

A period of rest of an hour was allowed for full recovery from the irritation caused by the cut to which the specimen was subjected, as also from the effect of rough handling in the process of mounting it in the apparatus. The specimen,



FIG. 88.

FIG. 88. Electric responses of two different specimens of procumbent branch of *Impatiens*; the direction of the responsive current is from the lower unexposed to the upper exposed side.

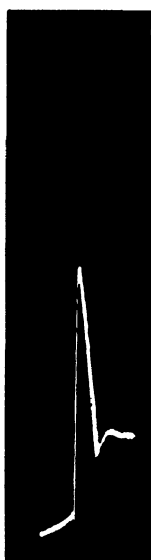


FIG. 89.

FIG. 89. Similar electric response of procumbent stem of *Ipomoea*.

it is to be noted, was placed in a moist chamber to prevent rapid drying.

The specimen being now subjected to vibrational stimulus, exhibited an electric response, the direction of which, across the tissue, was from the lower to the upper side, proving that the unexposed lower side was relatively the more excitable. The results prove that anisotropy of the organ has been induced by the unilateral action of light.

Records obtained with two different specimens of *Impatiens* are reproduced in fig. 88.

Experiment 122. *Electric response of procumbent stem of Ipomoea reptans*.—The upper sun-exposed side was, as usual, darker in colour. On subjecting the specimen to torsional vibration, the electric response (fig. 89) showed that in this case also the responsive current flowed from the lower to the upper side, proving the greater excitability of that side.

Experiment 123. *Electric response of fruit of Trichosanthes dioica*.—Trichosanthes is practically a creeping plant grown in vegetable gardens, the oblong fruit of which rests on the ground. As a result of exposure to sunlight from above, the upper side of the fruit is dark green, while the protected lower side is lighter in colour.

As it was difficult in this case to employ torsional vibration for the testing stimulus, the specimen was subjected first to local stimulus of a scratch successively applied on its lower and upper sides, and then to the intense diffuse stimulus of transverse section. The electric connections with the galvanometer were made with the upper and lower sides of the organ respectively. The two sides, as already stated, were at first separately stimulated by scratch stimulus.

I first describe the local effect of scratch stimulus on the lower and upper sides of the organ. When a point at the lower side of the fruit was scratched with a needle mounted on an insulating handle, the transmitted excitation reached the lower electric connection (the upper being at a considerable distance) and induced galvanometric negativity of the lower side, as seen in the up-curve of the first record in fig. 90. A point near the upper connection was next scratched, with the result of galvanometric negativity of the upper side (down-curve), as seen in the next record. The fruit was then subjected to transverse cut at a short distance from the two galvanometric connections. The intense excitation which was transmitted caused simultaneous stimulation of both the upper and lower sides of the fruit, the result being an up-response of considerable amplitude, as seen in the third record; the responsive

current was found to flow across the fruit from the unexposed lower to the exposed upper side, proving the lower to be relatively the more excitable.

In all the experiments described above, the horizontally laid specimens were subjected to the predominant differential stimulation by light; but the effect of gravity, as previously indicated, was not altogether absent. In order to prove that unilateral light by itself is effective in inducing anisotropy, the following experiments were carried out with different specimens which, growing in a vertical direction, were free from geotropic stimulation. Light, however, acted on them on one side only, bringing about differential stimulation of the two sides.

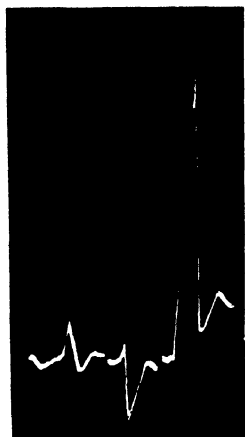


FIG. 90. Series of responses of *Trichosanthes dioica*.

Scratch stimulation of lower side gave rise to galvanometric negativity of that side (up-curve); that of upper side, galvanometric negativity of the same side (down-curve).

Transverse section induced resultant galvanometric negativity of the lower unexposed side as in the third record (up-curve).

Experiment 124. *Electric response of vertical Bryophyllum calycinum exposed to unilateral light*.—The vertical shoot was growing near a dark wall, and was therefore shielded from light on that side. The opposite side of the shoot was, however, exposed to sunlight for several days. The specimen, in the following experiment, was mounted vertically on insulated stands, the galvanometric connections being made respectively with the exposed and the unexposed sides. After a suitable period of rest for complete recovery from irritation caused during mounting,

diffuse stimulation of all sides of the organ was produced by transverse cut of the shoot at a short distance from the galvanometric connections. This diffuse stimulation was found to induce greater negativity of the shaded side, the direction of the current of response being from the shaded to the sun-exposed side. The organ had thus been

rendered anisotropic by differential action of light alone, the shaded side being relatively the more excitable.

Experiment 125. *Electric response of vertical flower-stalk of Crinum*.—As in the last case, the flower-stalk was protected on one side by a dark wall from the action of sunlight, which acted on the opposite side. Diffuse stimulation gave rise to an electric response (fig. 92), which showed that

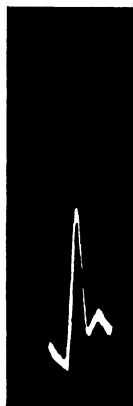


FIG. 91.

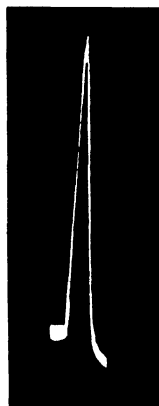


FIG. 92.



FIG. 93.

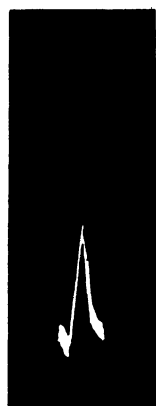


FIG. 94.

FIG. 91. Electric response of anisotropic shoot of *Bryophyllum*.

FIG. 92. Electric response of anisotropic flower-stalk of *Crinum*.

FIG. 93. Electric response of anisotropic leaf of *Aloe*.

FIG. 94. Electric response of anisotropic leaf of *Agave*.

In the records figs. 91-94 the direction of the current of response is from the shaded to the sun-exposed side of the organ, proving the shaded to be the more excitable side.

anisotropy had been induced by the differential action of light, and that it was the shaded side that was relatively the more excitable.

Experiment 126. *Electric response of leaf of Aloe perfoliata*.—The leaf was thick, and one side of it was alone exposed to the action of sunlight. Diffuse stimulation by transverse section gave rise to a resulting electric response (fig. 93), which showed that it was the shaded side of the leaf that had become galvanometrically negative, the responsive current flowing across the leaf from the shaded

to the exposed side. This proved that the shaded side was relatively the more excitable.

Experiment 127. *Electric response of leaf of Agave americana*.—The leaf, as in the last case, was exposed on one side to the strong action of sunlight, the opposite side being in the shade. Diffuse stimulation was found, as in the previous cases, to give rise to an electric response, the direction of which proved that the shaded side was the more excitable (fig. 94).

GEOTROPIC ANISOTROPY

A shoot is free from geotropic stimulation when it grows in the normal vertical direction. But when it is held in a horizontal position it exhibits, under geotropic action, growth-curvature which results in a bending upwards. The upper side becomes contracted and concave, while the lower side becomes expanded and convex. The already contracted concave side of the organ would obviously be less capable of further contraction. A diffuse stimulation would then be expected to induce differential excitatory contraction of the upper and lower sides of the organ. If this occurs, then an isotropic organ must have been rendered anisotropic under geotropic action.

The correctness of the above supposition was tested by two different methods of investigation, mechanical and electrical.

MECHANICAL DETECTION OF GEOTROPIC ANISOTROPY

I shall first describe the results given by the method of mechanical response.

Experiment 128. *Mechanical response of geotropically curved organ under diffuse stimulation*.—The geotropically curved *Bryophyllum* was subjected to diffuse stimulation by the passage of induction shocks from a secondary coil through the length of the organ. This caused a flattening of the curve by greater contraction of the lower side of the curved stem. The mechanical response under diffuse stimulation

was recorded by an Oscillating Recorder ; it shows an up-curve (fig. 95), due to relatively greater contraction of the lower or convex side of the organ, proving that side to be the more contractile and excitable. The organ recovered after cessation of the diffuse electric stimulation.

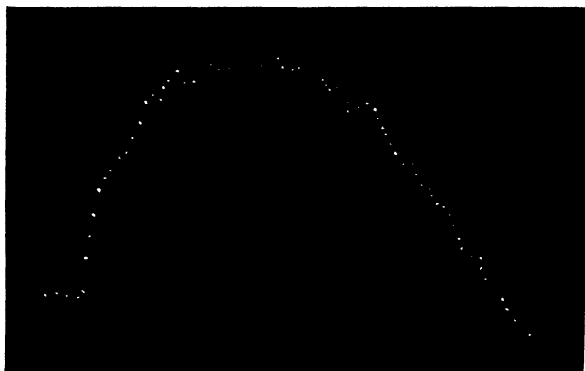


FIG. 95. Mechanical response of geotropically curved shoot of *Bryophyllum*, under diffuse electric stimulation.

Up-curve represents greater contraction of the lower convex side. Recovery completed in 25 minutes after cessation of stimulation.

ELECTRICAL DETECTION OF GEOTROPIC ANISOTROPY

The anisotropy induced by geotropic stimulus was next investigated by means of the independent method of electric response. The problem was studied first by demonstrating that there was no resultant electric response exhibited by an isotropic organ, that is to say, one which had not been previously subjected to any differential stimulation on its two opposite sides ; and then by discovering the specific electric response of an organ that had been subjected to geotropic stimulus.

Experiment 129. *Electric response of an isotropic organ.*—An erect shoot of *Bryophyllum* was employed for this experiment. It was growing in a greenhouse in which light was admitted only from the top, so that all the sides of the shoot were equally illuminated. Hence there was no differential

stimulation of its opposite sides by light ; the organ was also free from geotropic stimulation since it grew erect.

Electric connections with the galvanometer were made

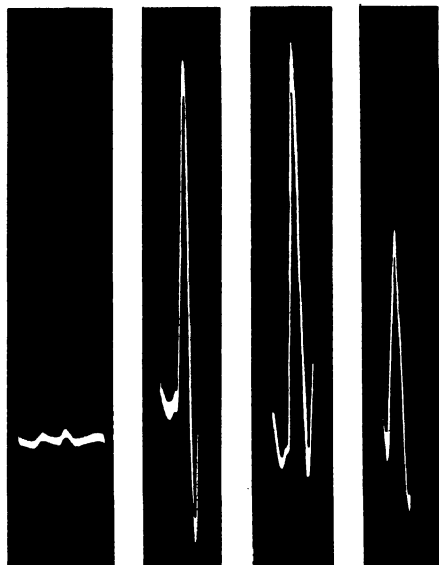


FIG. 96. FIG. 97. FIG. 98. FIG. 99.

FIG. 96. Vertical shoot of *Bryophyllum* exhibiting no resultant electric response on diffuse stimulation.

This indicates equal excitability of all sides of the organ, proving it to be isotropic.

FIG. 97. Electric response on diffuse stimulation of a horizontally laid shoot of *Bryophyllum* previously kept in a dark room, and therefore free from differential action of photic stimulus.

Up-response indicates galvanometric negativity of the convex side, which is therefore the more excitable.

FIG. 98. Electric response to diffuse stimulation of geotropically curved flower-stalk of *Tuberose*, the convex side being the more excitable.

FIG. 99. Similar electric response of geotropically curved flower-stalk of *Anthurium*.

in the usual manner with two opposite sides of the organ. Diffuse stimulation by a transverse cut, made at a short distance from the galvanometric contacts, did not give rise to any resulting deflection of the galvanometer, on account

of the equal excitatory reactions on opposite sides cancelling each other (fig. 96). This indicated that there was no anisotropy, the induction of which only occurs under differential stimulation. The following experiments demonstrate the effect of geotropism in inducing anisotropy.

Experiment 130. *Electric response of geotropically curved shoot of Bryophyllum*.—A potted *Bryophyllum* plant, growing vertical, was taken into a dark room and laid horizontally. Being in the dark, it was completely protected from the action of light, but was, nevertheless, subjected to pure geotropic stimulus, under whose differential action on the upper and lower sides the organ curved upwards. The upper concave and the lower convex sides were next connected with the galvanometer in the usual manner. The length of the shoot was then subjected to induction shocks, and the resultant electric response under simultaneous stimulation of the two sides recorded (fig. 97). *The responsive current was found to flow across the shoot from the convex to the concave side. This proved that anisotropy is induced under geotropism, the convex side of the organ being rendered the more excitable.*

Experiment 131. *Electric response of geotropically curved flower-stalk of Tuberose*.—In order to prove that the electric response of a geotropically curved organ is not characteristic only of stems but also of other radial organs, the flower-stalk of *Tuberose* was subjected to geotropic action in a dark room. Diffuse stimulation by induction shocks gave rise to a very strong electric response (fig. 98), which showed that it was the lower convex side that was relatively the more excitable.

Experiment 132. *Electric response of geotropically curved flower-stalk of Anthurium*.—The result recorded was precisely similar to those obtained with other specimens already described. Under diffuse stimulation the direction of responsive electric current was from the lower convex to the upper concave side of the organ (fig. 99), which fully confirmed the previous results.

The experiments described prove that an organ originally isotropic is rendered anisotropic under geotropic action, the lower convex side being rendered the more excitable.

SUMMARY

An organ is said to be *isotropic* when it is equally excitable on all its sides. The responses, mechanical or electrical, to diffuse stimulation, being equal on all sides, will then cancel each other. An isotropic organ is therefore found to exhibit no resultant mechanical or electrical response.

There are, however, other organs which, under diffuse stimulation, exhibit a resultant mechanical or electrical response. This is due to unequal excitability of its opposite sides, such an organ being therefore *anisotropic*. Natural anisotropy is found in the pulvinus of *Mimosa* and in tendrils of different plants.

In the naturally anisotropic pulvinus of *Mimosa* the lower half of the pulvinus is the more excitable, and on diffuse stimulation the greater contraction of the lower half gives rise to the resultant mechanical response exhibited by the fall of the leaf. Corresponding to the mechanical response is the electrical response of the anisotropic organ. Diffuse stimulation gives rise to greater galvanometric negativity of the more excitable lower half, the resultant responsive current flowing in the organ from the more excitable lower to the less excitable upper half of the pulvinus. The direction of the responsive current thus indicates the more excitable side of an anisotropic organ. Similar effects are observed in the naturally anisotropic tendrils of different plants.

Anisotropy is also induced in an isotropic organ by differential action of stimulus on its opposite sides. Such anisotropy induced by the differential action of light is detectable by the application of the Law of Electric Response, which has been established, namely, *that diffuse stimulation of an anisotropic organ gives rise to a resultant responsive current, which flows from the more to the less excitable side.* Thus in a vertical radial organ which has not been subjected to differential action of light, diffuse stimulation gives rise to no resultant electric response, on account of the organ being isotropic. But when one side of the organ is subjected to prolonged unilateral action of light, that side becomes

fatigued, with the resulting diminution of excitability. This induced anisotropy can be detected by the application of diffuse stimulus, when a responsive current is found to flow from the shaded to the exposed side, proving the shaded to be the more excitable side.

An isotropic organ is also rendered anisotropic by the stimulus of gravity. This is proved by the results of both mechanical and electric response to diffuse stimulation.

The mechanical response to diffuse stimulation is shown by the induced flattening of the geotropically curved organ, due to the greater contraction of the lower convex side, which is therefore the more excitable.

This conclusion finds independent support from the result of electric investigation on the effect of diffuse stimulation. The resultant current of response of a geotropically curved organ is found to flow from the lower convex to the upper concave side, proving the induction of relatively greater excitability of the convex side.

The anisotropy induced in an isotropic organ by differential action of stimulus on its opposite sides can thus be definitely detected by the method of electric response.

X.—INVESTIGATIONS ON THE ACTION OF DIFFERENT RAYS OF LIGHT AND OF ELEC- TRIC STIMULATION ON GROWTH

BY

A. GUHA-THAKURTA, C.S.AG.C.

THE results of extensive series of investigations, which had been carried out in this Institute, led to the conclusion that the variation of growth induced by photic stimulation is modified : (1) by the quality or colour of light ; (2) by the tonic condition of the growing organ ; and (3) by the point of application of stimulus, constituting direct or indirect stimulation.¹ The presence of numerous factors of variation therefore greatly complicates the results. In order to obtain further knowledge, I undertook to continue investigations on the subject with diverse plants at different seasons of the year and under varying tonic conditions.

The following investigations were carried out on the effect of different coloured lights under varying tonic conditions of the plant. A parallel series of investigations was also undertaken to determine the effect of an altogether different mode of stimulation, namely, the electrical. Finally I undertook the study of the effect of direct and indirect photic stimulation on growth. The subject will be treated in the following order :

A.—Modification of growth of vigorous plants under light.

- (1) Effect of white light.
- (2) Effect of blue light.
- (3) Effect of red light.
- (4) Effect of yellow light.

¹ Bose, *Life Movements in Plants* (1919), p. 210 ; *Growth and Tropic Movements of Plants* (1929), pp. 79, 86.

B.—Modifying influence of tonic condition on responsive variation of growth.

- (1) Effect of white light on growth of subtonic plants.
- (2) White light on plants kept in darkness.
- (3) White light on growth at standstill.
- (4) Pulsations in growth.
- (5) Coloured lights on subtonic plants.
- (6) Coloured lights on growth at standstill.

C.—Effect of electric stimulation on growth.

- (1) Effect on normal growth.
- (2) Effect on subtonic specimens.
- (3) Effect on growth at standstill.

D.—Effect of direct and indirect photic stimulation on growth.

- (1) Effect of indirect stimulation.
- (2) Alternate effects of indirect and direct stimulation.

RECORD OF GROWTH

The High-Magnification Crescograph.—For general purposes of investigation the Automatic High-Magnification Crescograph, devised and constructed in the Institute, has been employed; in this the magnification is secured by means of a compound system of two levers. The plant is attached to the short arm of a lever, the long arm of which is attached to the short arm of the second lever. If the magnification by the first lever be m , and that by the second n , then the total magnification is mn . It is thus possible to obtain a total magnification of six thousand to ten thousand times. An obstacle in obtaining accurate record of the curve of growth arises from the friction of contact of the bent tip of the writing lever with the recording smoked surface of glass; to obviate this the glass plate is made to oscillate to and fro, at right angles to the tip of the writing lever, the oscillations being at definite short intervals. The record of growth thus consists of a series of dots, and the intervening distance represents magnified growth during the short interval.

The Balanced Crescograph.—A far more sensitive method is that of the method of balance, by which the enhancement or retardation of the rate of growth, induced by external agents, is instantly exhibited. In this method the upward movement of growth is compensated by a corresponding subsidence of the plant, the tip of which is attached to the High-Magnification Crescograph. In this condition of balance the record remains horizontal; the exact rate of growth is found from the reading of an index against a dial.

The effect of an external agent is detected from the upsetting of the balance, either upwards or downwards. An up-curve represents an acceleration above, and a down-curve a depression below the normal rate of growth.¹

THE METHOD OF EXPERIMENTATION

For observing the effect of photic stimulation a horizontal beam of strong light, obtained from an arc lamp, was incident on the plant; two inclined mirrors were placed close behind, so that light acted on the plant from all sides. A glass trough with parallel sides, filled with alum solution, was interposed in the path of light so as to absorb the heat rays. For studying the effects of different rays of light on growth, coloured filters were employed, which transmitted blue, red, and yellow light. The average wave-length of blue was $440\text{ m}\mu$; of red $700\text{ m}\mu$; and of yellow light $610\text{ m}\mu$.

A.—MODIFICATION OF GROWTH OF VIGOROUS PLANTS UNDER LIGHT

The first series of investigations was undertaken with various species of young plants, such as *Cajanus indicus*, *Pisum sativum*, *Vicia Faba*, and *Crotalaria juncea*, all in a vigorous condition of growth. The specimens were kept in darkness for two hours before the commencement of the experiment, and the balanced record obtained in darkness. The rates of growth in diverse species of plants are obviously not the same, necessitating different adjustments for securing the balance.

¹ Bose, *Growth and Tropic Movements of Plants* (1929), pp. 24, 27.

I. EFFECT OF WHITE LIGHT

Experiment 133. *Effect of ordinary light on Cajanus indicus*.—The specimen, mounted on the Balanced Crescograph, gave, under condition of balance, a horizontal record. From the reading of the index, the absolute rate of growth was found to be 0.40μ per second. Application of white light of sub-maximal intensity at arrow upset the balance

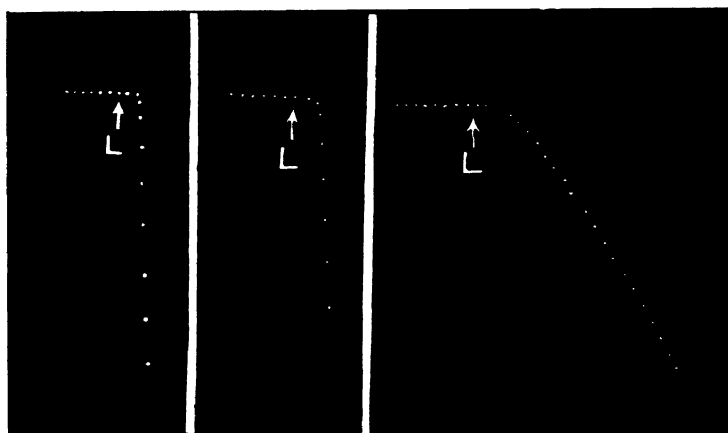


FIG. 100.

FIG. 101.

FIG. 102.

FIG. 100. Effect of white light on growth of *Cajanus* in vigorous condition.

The horizontal record at the beginning is taken under the condition of balance. Application of stimulus of light at L induces an upsetting of balance downwards, indicating a retardation of the rate of growth.

FIG. 101. Similar effect of stimulus of white light on growth of *Pisum*.

FIG. 102. Parallel effect of stimulus of white light on growth of *Crotalaria*.

downwards, indicating a retardation of the rate of growth (fig. 100). The latent period for the induced variation was found to be about 20 seconds.

Experiment 134. *Effect of ordinary light on Pisum sativum*.—The effect of white light of sub-maximal intensity in normal retardation of growth of *Cajanus*, has already been demonstrated in the last experiment. In the present

different plant, namely, *Pisum*, was employed, its rate of growth being 0.25μ per second. The record was, as usual, taken under the condition of balance; application of light at L induced a very marked retardation of growth, shown by a down-curve (fig. 101).

Experiment 135. *Effect of light on growth of Crotalaria juncea*.—After securing the exact balance, the absolute rate of growth was found to be 0.13μ per second. Light applied at L caused a down-curve which was not so abrupt as in the two previous cases (fig. 102). This indicated that the induced retardation was relatively less pronounced.

A very large number of experiments, carried out with different species of plants, fully support the results described in 'Growth and Tropic Movements of Plants,' that the effect of sub-maximal stimulation by white light is a retardation of the rate of growth of plants in a favourable tonic condition.

Turning next to the action of different rays of light on growth, the three principal constituents of white light, the blue, the red, and the yellow, may conspire with each other and induce a similar effect, namely, a retardation of growth; or one of these constituents may induce a contrary reaction. The following investigations were undertaken to obtain a clear answer to this question. For determining the results with the greatest accuracy it was necessary to raise the sensitivity of the Method of Balance to as high a degree as possible and thus obtain the individual effects of different coloured lights.

The results already described in 'Growth and Tropic Movements of Plants' show that, while blue light is most effective in retardation of the rate of growth, red and yellow lights are practically ineffective. The experiments described below are continued on similar lines.

2. EFFECT OF BLUE LIGHT

Experiment 136. *Effect of blue light on Cajanus*.—The plant was exactly balanced and its absolute rate of growth was found to be 0.16μ per second. Application of blue

light induced a marked retardation in the rate of growth, as shown by the down-curve in the record (fig. 103).

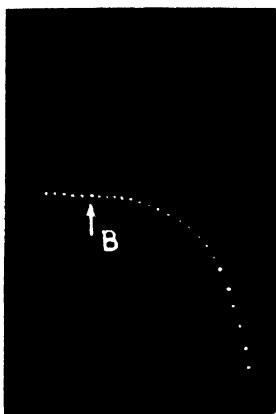


FIG. 103.

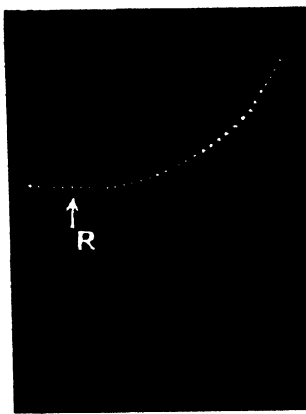


FIG. 104.

FIG. 103. Effect of blue light on growth of vigorous specimen of *Cajanus*.

Record taken under condition of balance. Application of blue light at B induces an upsetting of balance downwards, indicating a retardation of growth.

FIG. 104. Effect of red light on a vigorous specimen of *Cajanus*.

Application of red light at R causes an upsetting of balance upwards, indicating an acceleration of growth.

3. EFFECT OF RED LIGHT

The previously established fact, that red light had practically no modifying effect on growth, is now fully confirmed in a large number of experiments which I carried out with different plants. But in certain exceptional cases, as in the following, red light is found to exert a very definite effect on growth.

Experiment 137. *Effect of red light on growth of Cajanus*.—The plant in this case was exceptionally sensitive, and it responded to red light by an *acceleration* of growth. This unusual effect is seen recorded in fig. 104, taken under the condition of balance. The absolute rate of growth was 0.12μ per second. The incidence of red light at arrow

induced, as already stated, an enhancement of the rate of growth, exhibited by the up-curve, the acceleration occurring in the course of about 30 seconds.

4. EFFECT OF YELLOW LIGHT

The results previously obtained showed that yellow light was ineffective in inducing any variation of growth. In the following experiment the relative effects of yellow and blue lights were studied on an identical specimen.

Experiment 138. *Contrasted effects of yellow and blue lights.*—The experimental specimen was *Cajanus*, the absolute rate of growth of which was 0.14μ per second. A balanced record was taken and yellow light applied at Y ; this induced no change in the rate of growth. In order to demonstrate that the observed result was due, not to the insensitiveness of the particular specimen, but to the ineffectiveness of the yellow light, blue light was applied at the second arrow B, when the resulting retardation of growth was markedly exhibited by the down-curve (fig. 105).

Experiment 139. *Alternate effect of red and blue light on the same specimen.*—The absolute rate of growth of a very vigorous specimen of *Cajanus* was 0.25μ per second. After obtaining balance, red light was applied at the first arrow R ; the upset of the balance in an upward direction indicated that it had induced an acceleration of the rate of growth. Blue light was next applied in addition to the red, at the second arrow, with the result that its predominant opposite effect not only nullified the enhancement of the rate of growth caused by red light, but actually reversed it into a retardation (fig. 106).

It is thus seen that out of the two components of white light, blue and red, the former induces a retardation and the latter, at least in some cases, an acceleration of growth, their individual effects being antagonistic to each other. From this it would appear that blue light would, in some cases at least, prove to be more effective in inducing a retardation of growth than the unfiltered white light.

The recent results fully confirm those previously obtained,

that the variation of growth induced by visible radiation depends on the quality or colour of light. Blue light is most effective in inducing a retardation in the rate of growth, the yellow being quite ineffective. The same may be said

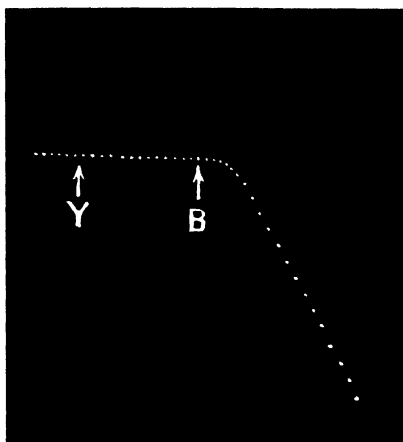


FIG. 105.

FIG. 105. Contrasted effects of yellow and blue lights on normal growth of *Cajanus*.

Record taken under condition of balance. Yellow light *y* induces no change, while blue light *b* induces a retardation of growth.

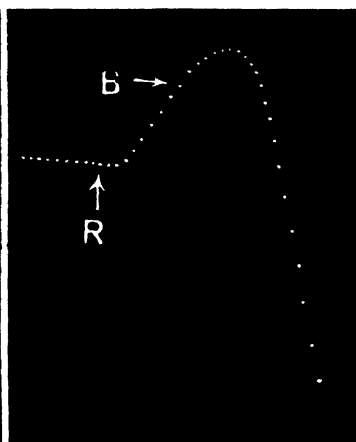


FIG. 106.

FIG. 106. Alternate effect of red light *r* and blue light *b* on growth of vigorous specimen of *Cajanus*.

The acceleration of growth under red light was reversed into retardation after application of blue light.

of red light, which, generally speaking, is ineffective ; but in certain exceptional circumstances it induces an acceleration of the rate of growth.

B.--MODIFYING INFLUENCE OF TONIC CONDITION ON RESPONSIVE VARIATION OF GROWTH

A very large number of typical experiments, which I have carried out with different species of plants under diverse conditions, fully support the results previously obtained, that the response is modified in a definite way by variation in the tonic condition of the specimen.

The subtonicity may have been brought about : (a) by conditions which cause a natural depression in the rate of growth ; (b) by long maintenance of the plant under the unfavourable condition of darkness ; and (c) by old age. The subtonicity in certain cases may be so pronounced that the rate of growth is extremely feeble or even at a standstill.

I. EFFECT OF WHITE LIGHT ON SUBTONIC SPECIMENS

Experiment 140. *Effect of white light on subtonic Cajanus.*—The growth of this specimen was very feeble, the rate being 0.09μ per second. The record was taken

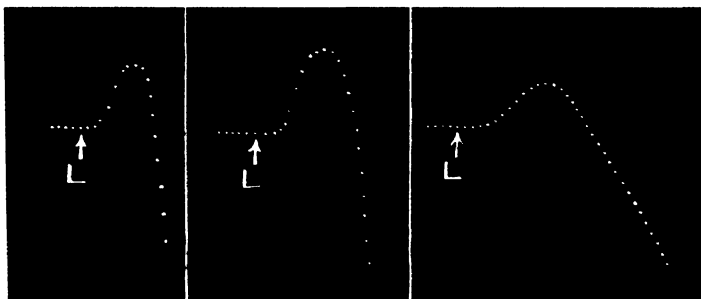


FIG. 107.

FIG. 108.

FIG. 109.

FIG. 107. Effect of white light on growth of subtonic *Cajanus*. Record taken under condition of balance. Light applied at L induces a preliminary acceleration followed by retardation of growth.

FIG. 108. Similar effect of white light on growth of subtonic *Pisum*.

FIG. 109. Parallel effect of white light on growth of subtonic *Crotalaria*.

under condition of balance, and light applied at L ; this caused an upset of balance in an upward direction (fig. 107), indicating an enhancement of the rate of growth. But after continued action of the light for about a minute, the preliminary acceleration was transformed into the usual retardation (shown by the down-curve), this being the characteristic response of an organ in the normal tonic

condition. The abnormal acceleration of growth under light will for convenience be designated as *positive*, while the normal retardation will be described as *negative*.

It would thus appear that the stimulus of light itself has raised the tonicity of the organ from below to above par, thus transforming the response from the abnormal positive to the normal negative.

Further experiments with different varieties of plants in a condition of subtonicity were carried out under the action of white light, typical examples of which are given below.

Experiment 141. *Effect of white light on subtonic Pisum.*—The absolute rate of growth of the specimen was 0.08μ per second. The first part of fig. 108 shows the record under balance; light was then applied at L, which induced a preliminary enhancement in the rate of growth. Continued action of light for about a minute and a half, however, transformed the abnormal positive response of acceleration into the normal negative response of retardation of growth. This result is essentially similar to that in the last case.

Experiment 142. *Effect of white light on subtonic Crotalaria.*—The rate of growth was 0.05μ per second. The immediate effect of light was an acceleration; but the continued action of light converted, as in previous cases, the abnormal positive into normal negative response (fig. 109).

2. WHITE LIGHT ON PLANTS KEPT IN DARKNESS

The subtonicity was, in the following cases, artificially induced by prolonged maintenance of the plant under the unfavourable condition of deprivation of light. Different specimens of plants, namely, *Cajanus*, *Pisum*, and *Vicia Faba*, were thus brought into a state of subtonicity.

Experiment 143. *Effect of white light on growth in plants long maintained in darkness.*—Though plants kept for a considerable length of time in darkness still exhibit growth, yet their tonicity is depressed below par. The results of the effect of light on the three species of plants that had been kept in prolonged darkness are given in figs. 110, 111, and

112. The first portion of the curve shows the horizontal record under condition of balance. In these three cases the balance was found to be upset for a considerable length of time in an upward direction under the application of light at L, the up-curve, as previously explained, indicating an acceleration of the rate of growth.

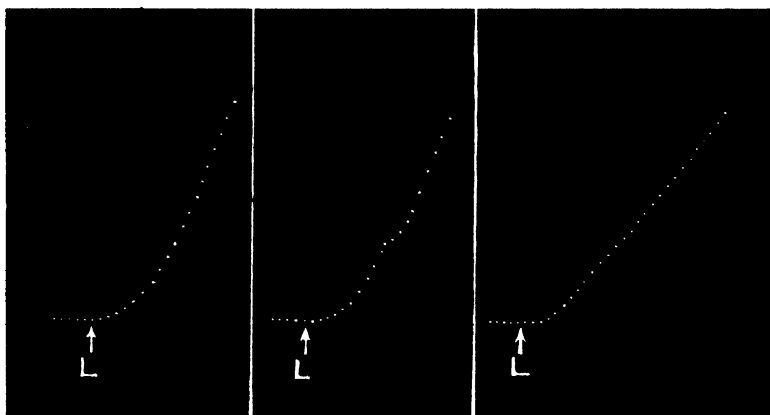


FIG. 110.

FIG. 111.

FIG. 112.

FIG. 110. Effect of white light on organs in which subtonicity is induced by prolonged deprivation of light (*Cajanus*). Record under condition of balance. Continued action of light applied at L induces enhancement of rate of growth, which persists for a long time.

FIG. 111. Similar effect of white light on subtonic *Pisum*.

FIG. 112. Parallel effect of white light on subtonic *Vicia Faba*.

The various results described prove that in organs in a condition of subtonicity which is either natural or artificially induced, the action of white light is an enhancement instead of the normal retardation of the rate of growth.

3. WHITE LIGHT ON GROWTH AT A STANDSTILL

Growth in plants may come to a state of standstill on account of excessive subtonicity ; this may be described as an atonic condition. Specimens which are comparatively old often exhibit this state of arrested growth. In the following experiments growth was found to be at a standstill.

Experiment 144. *Effect of light on growth at a standstill in Cajanus.*—On account of the arrested growth the record was horizontal, it being unnecessary to secure the preliminary balance. Application of strong light for less than a minute at L (fig. 113) produced the very striking result of revival of growth, as shown by the sudden erection of the curve. In previous cases of moderate subtonicity (Experiments 140–142), the positive acceleration was transformed into negative retardation under continued action of the

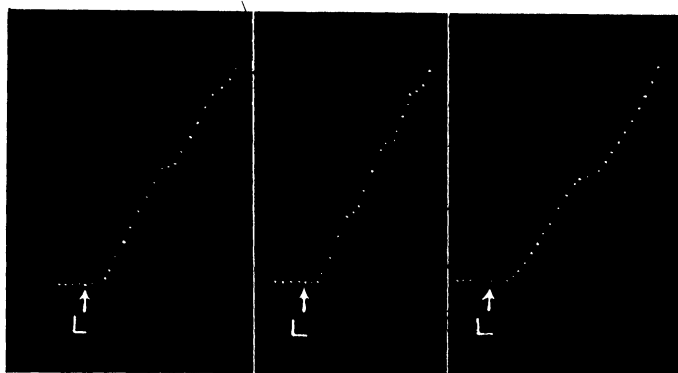


FIG. 113.

FIG. 114.

FIG. 115.

FIG. 113. Effect of white light on revival of growth in *Cajanus*. Horizontal record without balance indicates growth at a standstill. Revival of growth by application of light at L. Note pulsations in growth.

FIG. 114. Similar effect of light in revival of growth in *Pisum*.

FIG. 115. Parallel effect of light in revival of growth in *Crotalaria*.

stimulus. In the present case, however, the subtonicity was so excessive that the positive response by acceleration from zero growth persisted even under the prolonged action of light.

Experiment 145. *Revival of growth in Pisum.*—A very similar result was obtained with *Pisum*, in which growth was at a standstill. Application of light at L caused a renewal of growth, as exhibited by the sudden erection of the curve (fig. 114).

Experiment 146. *Revival of growth in Crotalaria.*—In this specimen the rate of growth was also in a state of

standstill. Application of light at L induced a revival of growth, as seen in the record (fig. 115).

4. PULSATIONS IN GROWTH

It is interesting to note that in the three experiments just described, the records of revived growth exhibit a number of pulsations, lending support to the conclusion previously arrived at, and described elsewhere, that the growth itself may be a pulsatory phenomenon. This was

TABLE XXV.—COMPARATIVE EFFECT OF LIGHT ON OPTIMUM, ON SUBTONIC, AND ON ATONIC SPECIMENS.

Specimen	Tonic condition	Rate of growth per second	Characteristic response
<i>Cajanus</i>	Optimum	0.40 μ	Negative
	Subtonic	0.09 μ	Positive followed by negative
	Atonic	<i>nil</i>	Positive
<i>Pisum</i>	Optimum	0.25 μ	Negative
	Subtonic	0.085 μ	Positive followed by negative
	Atonic	<i>nil</i>	Positive
<i>Crotalaria</i>	Optimum	0.19 μ	Negative
	Subtonic	0.05 μ	Positive followed by negative
	Atonic	<i>nil</i>	Positive

found specially in cases where the zone of growth was narrow and circumscribed ; but when it was of considerable length then the record did not show any marked pulsations. The probable explanation of this is that the phase of maximum growth is not the same in the different zones of growth ; it may thus happen that the maximum rate of growth at any

particular zone corresponds to the minimum rate in another zone, with the probable result that the average rate remains approximately the same. But in the cases recorded (figs. 113, 114, 115), the growth being previously at a standstill, light initiated growth in all the different sections at about the same time. Any phasic variation, a maximum followed by a minimum rate, would thus appear to have simultaneously occurred in all the sections, and was exhibited as pulsations in growth.

The modifying influence of tonic condition on the sign of response is clearly demonstrated by the typical results given in Table XXV.

5. COLOURED LIGHTS ON SUBTONIC PLANTS

Investigations were next carried out on the effects of different coloured lights on diverse species of plants, which had been brought to a condition of subtonicity by having been kept in prolonged darkness.

Experiment 147. *Effect of blue light on growth of subtonic plants.*—The specimen *Cajanus* had been kept in darkness for a considerable length of time, being thereby brought to a condition of subtonicity. After obtaining exact balance of growth, blue light of the wave-length $440\text{ }\mu$ was applied to the growing organ. The effect induced by blue light is seen in the record given in fig. 116, in which the balance is upset in an upward direction, indicating an enhancement of the rate of growth.

Experiment 148. *Effect of alternate application of red and blue lights on subtonic specimen.*—Subtonicity was induced in the plant *Cajanus*, as in the previous case, by keeping it in darkness for several days. After obtaining a balanced horizontal record, red light was applied at R, and continued for a considerable length of time. Under red light there was no upsetting of the balance, proving it to be ineffective in inducing any change of growth in the subtonic specimen. Blue light was next applied at B, with the result that there was an acceleration of the rate of growth, as seen in the upsetting of the balance upwards (fig. 117).

The result of this experiment proves that red light has no effect in inducing variation in the rate of growth in subtonic specimens. The acceleration of growth under red light, described in a previous experiment, occurs only in

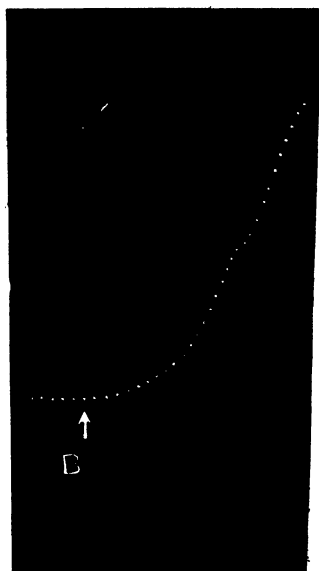


FIG. 116.

FIG. 116. Effect of blue light on growth of subtonic *Cajanus*. Record taken under condition of balance. Application of blue light at B enhances the rate of growth.

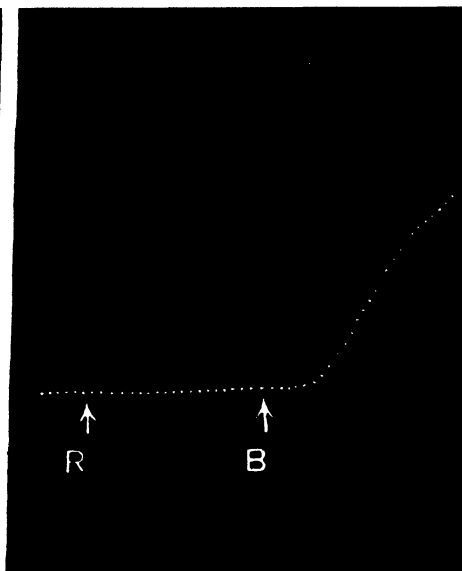


FIG. 117.

FIG. 117. Effect of alternate application of red and blue lights on subtonic specimen (*Cajanus*).

Record taken under condition of balance. Application of red light at R induces no variation in growth, while application of blue light at B causes an enhancement in the rate of growth.

the special circumstances of exceptionally vigorous and sensitive condition of the specimen.

The effect of blue light on subtonic specimens is similar to that of white light in inducing an acceleration instead of the normal retardation of growth (*cf.* figs. 110, 111, 112).

6. COLOURED LIGHTS ON GROWTH AT A STANDSTILL

The following experiments were next undertaken on plants whose condition of subtonicity was so excessive as to have resulted in an arrest of growth.

Experiment 149. *Effect of blue light on growth at a standstill.*—The pair of records of two different species of plants,

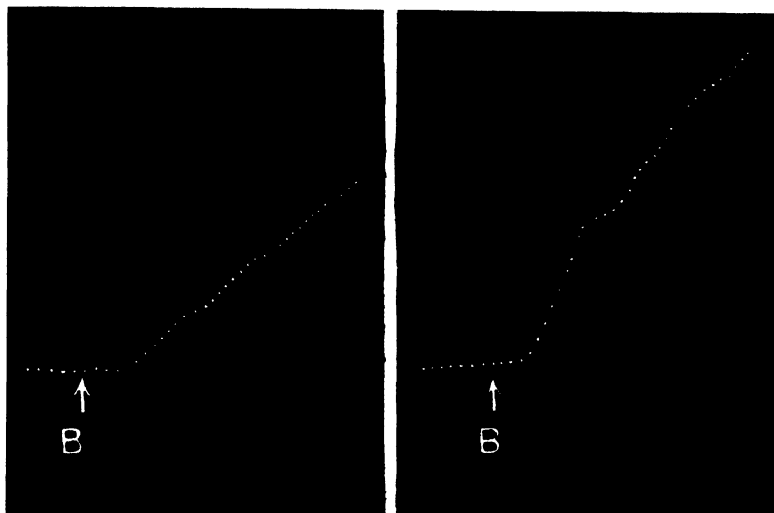


FIG. 118.

FIG. 119.

FIG. 118. Effect of blue light on growth at a standstill (*Cajanus*). Horizontal record without balance indicates arrested growth. Application of blue light at B causes a revival of growth.

FIG. 119. Similar effect of blue light in the revival of growth in *Pisum*.

Cajanus and *Pisum*, taken without balance, were found to be horizontal since growth was at a standstill. On application of blue light at B there was a revival of growth, shown by the sudden erection of the curves (figs. 118, 119). These records of revived growth also exhibited pulsations, as those under the action of white light on growth at a standstill (cf. figs. 113, 114, 115).

Experiment 150. *Effects of alternate application of red*

and blue lights on growth at a standstill.—A relatively old specimen of *Cajanus* was taken, in which growth was arrested. Red light was found to be quite ineffective in inducing any change; the subsequent application of blue light, however, was found to bring about a revival of growth.

In summarising the characteristic effects of different coloured lights on subtonic specimens, it may be stated that the results are similar whether the subtonicity is moderate or so excessive as to have caused an arrest of growth. Red light has no effect in inducing any change, whereas blue, like white light, induces an acceleration or revival of growth, in contrast with the normal retardation induced in vigorous specimens.

C.—EFFECT OF ELECTRIC STIMULATION ON GROWTH

The question next arises whether the characteristic effect of light on growth is due, in some hitherto unexpected way, to its photo-synthetic action, or whether it is essentially due to its acting as a stimulus. In solving this problem Sir J. C. Bose employed an altogether different mode of stimulation, namely the electrical: (1) on normal growth; (2) on growth of subtonic specimens; and (3) on growth at a standstill. The numerous experiments which I carried out on similar lines fully confirm his results.

Method of experiment.—A length of about 4 cm. of growing stem was electrically stimulated by induction shocks from a secondary coil. The intensity of the shock was adjusted by gradual approach of the secondary nearer to the primary coil. In this way a minimally effective stimulus was found. The duration of application of this stimulus was also maintained constant in successive experiments by a tapping key and a metronome, interposed in series with the primary coil.

The following experiments were carried out with different specimens of *Cajanus*, the only variation being in the condition of their tonicity.

I. EFFECT ON NORMAL GROWTH

Experiment 151. *Electric stimulation in modification of normal growth.*—A moderately vigorous specimen of *Cajanus* was mounted in the Balanced Crescograph; the absolute rate of growth was found to be 0.19μ per second. After obtaining the balanced record, stimulus was applied at S. This is seen to cause an upset of balance downwards, which indicates a retardation of growth (fig. 120).

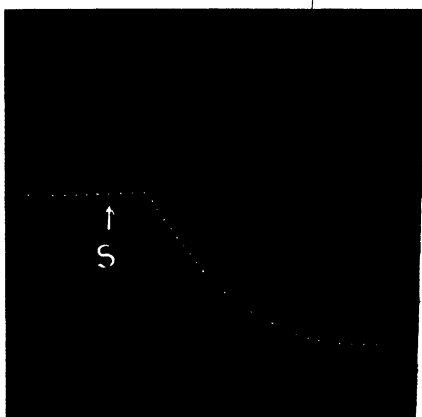


FIG. 120.

FIG. 120. Effect of electric stimulus on normal growth (*Cajanus*). Record taken under condition of balance. Application of electric stimulus at s induces a retardation in the rate of growth (down-curve).

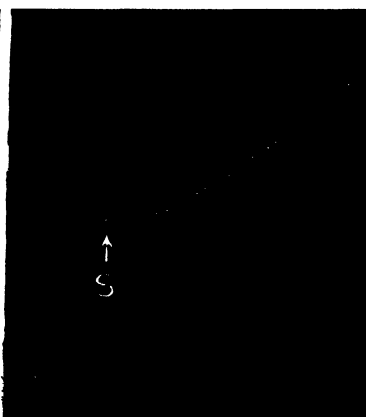


FIG. 121.

FIG. 121. Effect of electric stimulus on growth of subtonic specimen (*Cajanus*).

Record taken under condition of balance. Application of electric stimulus at s induces an acceleration in the rate of growth.

The effect of electric stimulus on normal growth is thus similar to that of photic stimulus, namely, a retardation of the rate of growth. This is true not only in the case of normal growth but also in cases of specimens in subtonic and atonic conditions as demonstrated in the following experiments.

2. EFFECT ON SUBTONIC SPECIMENS

Experiment 152. *Effect of electric stimulation on growth in a subtonic specimen.*—A specimen of *Cajanus* was brought to a condition of subtonicity after having been kept in darkness for several days. After obtaining the balance, electric stimulus was applied at S. This induced an up-setting of the balance upwards, indicating an acceleration of the rate of growth (fig. 121).

The modification of response of subtonic specimens under the electric stimulus is thus found to be precisely similar to that under the stimulus of light. The sign of response in subtonic specimens is seen to be modified from normal retardation to acceleration of the rate of growth.

3. EFFECT ON GROWTH AT A STANDSTILL

Experiment 153. *Effect of electric stimulus on arrested growth.*—The subject of this experiment was a specimen of

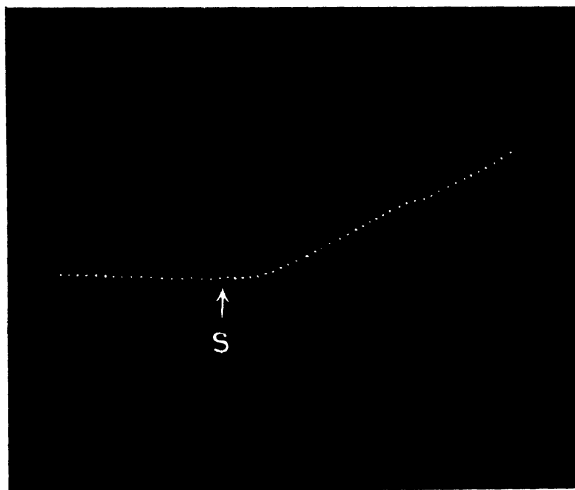


FIG. 122. Effect of electric stimulus in revival of growth at a standstill (*Cajanus*).

Horizontal record without balance indicates arrested growth. Application of electric stimulus at s brings about a revival of growth.

Cajanus in which growth had come to a state of standstill. The record, even without balance, was therefore horizontal. On application of the electric stimulus at S, growth became revived as shown by the sudden rise of the curve (fig. 122).

It is thus seen that the responsive variations of growth under changed tonic condition of the organ are exactly similar under electric and photic stimuli. Both modes of stimulation induce a retardation of growth in plants in a vigorous condition ; but in a condition of subtonicity they cause an enhancement of the rate of growth ; and, finally, growth at a standstill is revived by both. From these facts it would appear that the modifying influence of light on growth is not improbably due to its action as a stimulus.

D.—EFFECT OF INDIRECT AND DIRECT PHOTIC STIMULATION ON GROWTH

Indirect stimulation is effected by the application of stimulus at some distance from the responding region of growth ; *direct* stimulation, on the other hand, is brought about by the application of stimulus at the growing region itself.

Method of experiment.—The experimental specimen is held by a clamp a little below the region of growth. The plant, held in its stand, is suitably mounted on the platform of the Balanced Crescograph, and a horizontal record obtained after securing the exact balance. The stimulus of light is then applied, either at some distance below the region of growth, thus constituting indirect stimulation, or it is directly applied at the growing region itself. The upsetting of the balance in an upward or a downward direction would then indicate whether the change induced under indirect or direct stimulation is an acceleration or a retardation of growth.

In 'Growth and Tropic Movements in Plants' the effects of direct and of indirect *electric stimulation* on growth have been fully described. It was there shown that, while direct stimulation induces the normal retardation of the rate of growth, indirect stimulation gives rise to an enhancement

of the rate. The present investigations refer to the characteristic effects of indirect and of direct *photic stimulation* on growth.

I. EFFECT OF INDIRECT STIMULATION

Experiment 154. *Effect of indirect stimulation on growth of Cajanus.*—The plant, as already explained, was held by a clamp immediately below the region of growth, and a record under condition of balance was obtained. The

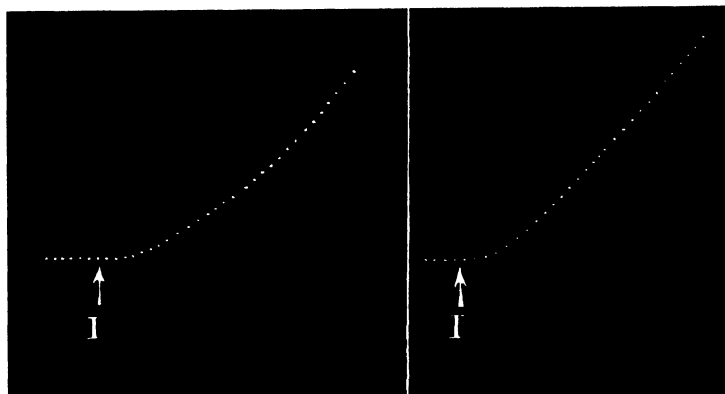


FIG. 123.

FIG. 124.

FIG. 123. Effect of indirect photic stimulation on growth of *Cajanus*.

Record taken under balance. Application of indirect stimulation at I induces an enhancement of the rate of growth.

FIG. 124. Similar effect of indirect photic stimulation on growth of *Pisum*.

absolute rate of growth was found to be 0.18μ per second. The stimulus of light was applied at I, 1 cm. below the clamp, thus constituting an indirect stimulation. The result is seen in fig. 123 in the erection of the curve indicating an acceleration of the rate of growth. The indirect effect of stimulus of light, as of electric stimulus, described in the previous paragraph, is thus an enhancement of the rate of growth.

Experiment 155. *Effect of indirect stimulation on growth of Pisum.*—A different species of plant, *Pisum*, was used in this experiment. The result was essentially similar to that in the last case. The rate of growth in this specimen was 0.17μ per second. Indirect stimulation induced a marked enhancement of the rate of growth (fig. 124).

2. ALTERNATE EFFECTS OF INDIRECT AND DIRECT STIMULATION

The following experiments were carried out on the effect of alternate application of indirect and direct stimulus on the same specimen.

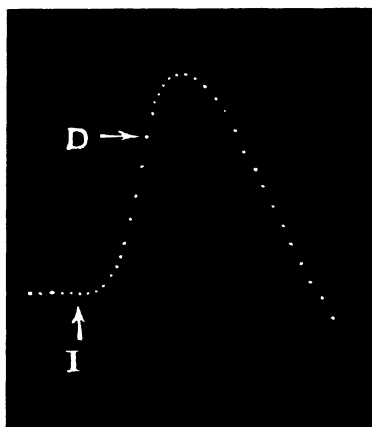


FIG. 125.

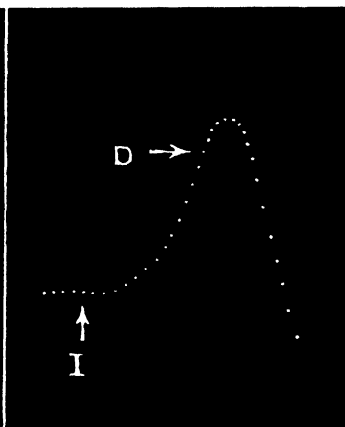


FIG. 126.

FIG. 125. Effect of indirect and direct photic stimulus on growth of *Cajanus*.

Horizontal record taken under balance. Indirect stimulus at vertical arrow induced an enhancement of the rate (up-curve). Direct stimulus at horizontal arrow D reversed acceleration into retardation (down-curve).

FIG. 126. Similar effect of indirect and direct stimulus on growth of *Pisum*.

Experiment 156. *Effects of indirect and of direct stimulation on growth of Cajanus.*—The rate of growth of the specimen was 0.25μ per second. After obtaining the

balanced horizontal record, the stimulation of light was indirectly applied at I ; the upsetting of the balance upwards indicates the resulting enhancement of the rate of growth. The application of light was next transferred to the growing region itself at the horizontal arrow D, the stimulation being now direct. This not only arrested the enhanced rate of growth but reversed it into normal retardation, which occurred in the course of about a minute. The acceleration under indirect stimulation (up-curve), reversed under direct stimulation (down-curve), is seen in fig. 125.

Experiment 157. *Effects of indirect and of direct stimulation on Pisum.*—The normal rate of growth of the specimen was 0.19μ per second. The record taken, under usual condition of balance, was horizontal (fig. 126). Indirect photic stimulation at I induced an acceleration of the rate of growth (up-curve). Direct stimulation at D, on the other hand, transformed the acceleration into normal retardation of growth (down-curve).

The action of stimulus is thus seen to be modified by the point of application, indirect stimulation inducing one effect, direct stimulation precisely the opposite.

SUMMARY

A plant in an optimum or vigorous condition of growth responds to the stimulus of white light by a retardation in the rate of its growth.

The response to visible radiations is modified by the quality or colour of the light. The yellow constituent of white light is wholly ineffective in inducing any change in the rate of growth. Red light, generally speaking, is also ineffective ; but certain highly sensitive specimens exhibit an acceleration of the rate of growth. Blue light, on the other hand, is highly effective in inducing the normal retardation of growth.

The sign of response of the growing organ to the stimulus of light is also modified by its tonic condition. When the plant is in an optimum condition the response is *negative*, or a *retardation* of the rate of growth.

In a slightly subtonic specimen the immediate effect of white light is a *positive* response by an *acceleration* of the rate of growth. The continued action of light is found to raise the tonicity to a condition of *par*, the abnormal positive being thereby converted into the normal negative. The characteristic response of a slightly subtonic specimen under continued action of light may therefore be described as a preliminary positive followed by a negative.

In regard to the effect of coloured lights on subtonic specimens, red light is ineffective; blue light, on the other hand, induces a positive variation, *i.e.* an acceleration instead of the normal retardation of growth.

A more pronounced condition of subtonicity is induced by long maintenance of the plant in darkness. The response of such an organ to the stimulus of light is an acceleration of growth or positive response which persists for a long time.

In extreme cases of subtonicity, also as the effect of age, the growth of the organ is brought to a state of stand-still. The arrested growth becomes revived under the stimulus of light, the sign of response being therefore positive.

The characteristic effects of different coloured lights are essentially similar in all subtonic specimens, whether the subtonicity is moderate or so excessive as to have caused an arrest of growth. Red light induces no change, whereas blue light, like white light, induces an acceleration or revival of growth.

The question whether the variation of growth induced by light is due purely to its photo-synthetic action, or whether it is due to its action as a stimulus, is answered by the discovery of the effect of a non-photic mode of stimulation, such as the electrical.

It is shown that under variations of tonic condition the characteristic responses of the organ to electric stimulus are exactly parallel to those under the stimulus of light. In normal condition both of these induce a retardation of growth; in the condition of subtonicity they cause an enhancement of the rate of growth; while growth at stand-still is revived by both of them. From these facts it would

appear highly probable that one of the important factors in the modification of growth by light is its action as a stimulus.

Growth is also modified by the point of application of the stimulus. Thus, while direct stimulation induces a retardation of growth, indirect stimulation enhances the rate.

XI.—EFFECTS OF EXTRACTS OF CERTAIN INDIGENOUS PLANTS ON FROG'S HEART

BY

GURU PRASANNA DAS, L.M.S.

THE investigations described in the present Paper were mostly carried out with extracts obtained from indigenous plants. In regard to their characteristic effects on the heart, a few general remarks may be made about conditions which are likely to modify the reactions. The effect of the active substance present in the extract depends : (1) on the nature of the drug ; (2) on the dose and duration of application ; and (3) on the tonic condition of the heart. In regard to the modifying action of tonic condition on response, a vigorous heart may not show any marked change under the action of a stimulant, probably because a heart which naturally exhibits maximum activity cannot exhibit any further enhancement. The effect of a stimulant on a depressed heart is, on the other hand, very strikingly exhibited by an increase of activity.

There are other conditions also, such as habitat and season, which modify the effectiveness of the drugs obtained from plants. Thus, investigations carried out on the subject at the Bose Research Institute showed that the proportion of mineral constituents in the ashes of a particular species of plants, as also the pH value of the extracts, were markedly different in two specimens, one grown in the Experimental Garden of the Institute in Calcutta, and the other in the Physic Garden of the Institute at Falta on the Ganges. The amount of active substance present depends, moreover, on the season ; for it was found to vary in different specimens which were collected during the rainy and the dry seasons.

PREPARATION OF THE EXTRACT

One of the methods for obtaining the preparation from the plant is to extract the active substance with cold water. About 50 grams of fresh leaves, shoots, roots, flowers, fruits, or seeds, were bruised in a mortar and thoroughly mixed up with 500 c.c. of normal saline or Ringer solution. The mixture was strained through ordinary muslin and then filtered. The fresh solution thus obtained is found to remain effective for experiments carried out in the course of the same day.

As such a solution does not keep for more than 12 hours or so, it was necessary to employ a second method for securing the extract. According to this, the leaves, shoots, or other parts of plants, were dried in air for a few days and then powdered in a mortar. About 500 grams of this powder were boiled with 2 litres of distilled water in a flask, until it was reduced to 500 c.c. in volume. The fluid was then filtered and the filtrate evaporated on a water-bath till a pasty or dried solid mass was obtained. This mass was then placed in a stoppered bottle and preserved for future use. Its efficacy was found to remain unchanged for a considerable length of time. For studying the effect of a particular dose of the drug, the solid extract is redissolved in proper quantities of normal saline or Ringer solution and then directly applied on the heart.

THE CHARACTERISTIC EFFECT OF DRUGS

In regard to the specific action of a drug, it is said to cause stimulation when it increases the activity of the heart, while it is regarded as a depressant when it lowers the activity of the organ. From their characteristic action drugs may be divided into several classes : first, those which decrease the frequency but increase the force of the beat of the heart ; secondly, those which not only decrease the frequency but also the force of the heart-beat ; and finally there is an intermediate class in which the frequency undergoes either

an increase or a diminution, without much altering the force of the heart-beat.

The effects of extracts from certain plants, growing extensively in India, will be described in the following order :

- I. Ayapana (*Eupatorium Ayapana*).
- II. Jagat Madan (*Justicia Gendarussa*).
- III. Banafsha (*Viola odorata*).
- IV. Hattisura (*Heliotropium Indicum*).
- V. Gokshuri (*Tribulus terrestris*).
- VI. Chakulia (*Ukaria lagopoides*).

For an accurate study of the effect of a drug on the heart, an automatic method of record of great precision is essential. The ordinary Cardiograph labours under the disadvantage that the friction of the tip of the writing lever against the moving smoked-glass plate introduces an error in the correct record of the amplitude of pulsation. The old method, moreover, does not enable determination of the exact period of each individual pulsation. This difficulty has been overcome by the Resonant Cardiograph, devised by Sir J. C. Bose, which records the period as well as the phasic alternations of the heart-beat with unprecedented accuracy. The systolic contraction and its persistence, the diastolic expansion and the subsequent pause, and any variations of these under external agencies, can thus be determined in a quantitative manner.

THE RESONANT CARDIOGRAPH

A semi-diagrammatic representation of the recorder is given in fig. 127. The writing lever is a fine steel wire which is maintained in a state of resonant vibration, being exactly tuned to vibrate, say, 15 times in a second. It is supported on jewel bearings in the centre of one pole of an electro-magnet. The magnetising coil is in circuit with a storage battery and a vibrating reed V, seen to the left of the figure, which periodically completes the electric circuit. When the reed is exactly tuned to vibrate 15 times in a second, the recording writer is thrown into sympathetic

vibration and strikes the smoked-glass plate once in every fifteenth of a second. With finer recorders it is possible to measure as short a period as a thousandth part of a second. The beat of the heart is sometimes so very active that there is an overshooting of the movement of the recording lever. This is prevented by means of a damper, not shown in the figure, which consists of a mica vane attached to the short arm of the lever, and fully dipped into a vessel of water. The magnification employed for the present experiments

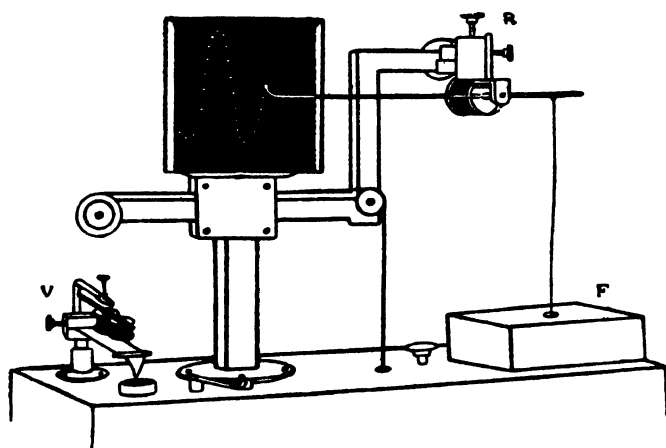


FIG. 127. The Resonant Cardiograph.

The writing lever is set in resonant vibration by the intermittent current produced by the vibrating reed *V*. *F*, the frog's chamber.

was 5 times, and the vibrating frequency of the writing lever, as already stated, was 15 times in a second. The perpendicular distance of the curve from the apex to the base gives a measure of the amplitude of pulsation, the intervening distance between successive dots representing one-fifteenth of a second. Hence it is possible to know the exact time of systolic contraction, and the duration of pause between successive pulsations, as well as the period of a complete pulsation. The different phases of the normal pulsation undergo definite variation under the characteristic action of a drug, from

which the stimulatory or depressing action of the drug can be inferred.

The heart of a pithed frog is exposed, and the apex is attached to the writing lever by means of a hook and thread. The specimen is placed in a frog-chamber kept in a humid condition. The plant-extract of a given concentration is then allowed to fall drop by drop directly on the heart, and the second row of the record gives indication of the effect induced within a short time of application. The subsequent rows show the effect of either a stronger dose or of prolonged application, as the case may be.

I. AYAPANA

Ayapana is the Bengalee name of the plant *Eupatorium Ayapana*. It belongs to the natural order *Compositae*. This plant, often confused with *Bishalyakarani*, is found naturalised in many parts of India.

Description.—A small shrubby plant, with reddish branches and lanceolate opposite leaves which are about 4 inches long and $\frac{3}{4}$ inch broad.

Uses.—Employed as an emetic, diaphoretic, antiperiodic, and purgative. The results of investigations to be described presently indicate that it is also a cardiac stimulant.

Chemical composition.—The inorganic constituents of the plant have been determined at the Bose Research Institute, and discovery made of the presence of a crystalline principle in the plant.

Preparation of the aqueous extract.—A semi-solid extract was made from dried leaves and shoots of *Ayapana*, collected from the Bose Research Institute Garden, Calcutta, during the months of January to March. The resulting extract was a reddish-brown scaly mass which deliquesces when kept for a few days. It has a sweetish taste and aromatic odour. Two solutions were made out of this extract : (1) a 2 per cent. solution ; and (2) a 6 per cent. solution. Modifications

of the physiological activity induced by these solutions were tested by direct application on the heart of medium-sized frogs.

Experiment 158. *Effect of a dilute dose of Ayapana.*—After obtaining a normal record of the heart-beat, a 2 per cent. solution of the extract of *Ayapana* was poured drop by drop on the heart, and after an interval of 5 minutes the second row of the record was taken. The amplitude of

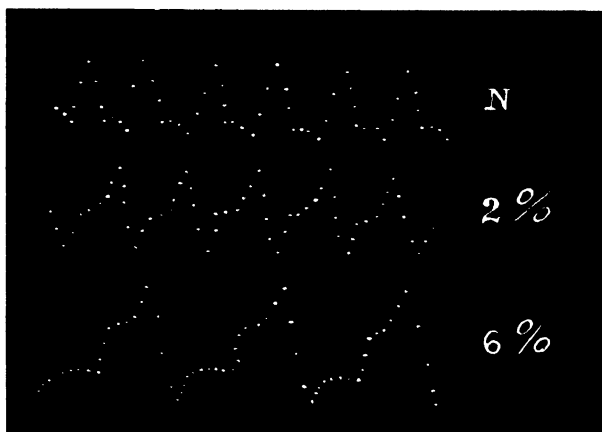


FIG. 128. Effect of different strengths of solution of *Ayapana* on frog's heart.

First row—typical normal record.

Second row—effect of 2 per cent. solution.

Third row—effect of 6 per cent. solution.

pulsation was found to become slightly increased, while the frequency of the beat became somewhat slower (fig. 128). Ten different specimens gave similar results.

Experiment 159. *Effect of stronger dose of Ayapana.*—The next series of experiments was carried out with a dozen frogs similar to the above, a stronger, 6 per cent., solution being directly applied on the heart. The results in every case showed a marked increase in the amplitude and a considerable slowing-down of the rate of pulsation (see third row, fig. 128).

The following table gives a summary of the typical

results obtained on the effects of different strengths of solutions on the amplitude and frequency of pulsation of the frog's heart :

TABLE XXVI.—EFFECT OF DIFFERENT STRENGTHS OF SOLUTION OF *AYAPANA* ON THE AMPLITUDE AND FREQUENCY OF PULSATION (FROG'S HEART).

Specimen	Applied solution	Magnified amplitude of pulsation in mm.	Frequency of pulsation
I	Normal 2 per cent. sol.	9 mm. 11 "	60 per minute 45 " "
2	Normal 2 per cent. sol.	11 mm. 12 "	53 per minute 41 " "
3	Normal 2 per cent. sol.	8 mm. 9 "	90 per minute 85 " "
4	Normal 2 per cent. sol.	9 mm. 11 "	90 per minute 75 " "
5	Normal 6 per cent. sol.	9 mm. 14.5 "	90 per minute 45 " "
6	Normal 6 per cent. sol.	10.5 mm. 15 "	90 per minute 37.5 " "

The results above summarised lead to the conclusion that *Ayapana* is a cardiac stimulant belonging to the class

of drugs which increases the force of the heart-beat, but diminishes its frequency.

II. JAGAT MADAN

The botanical name of the plant is *Justicia Gendarussa*. The plant is also known as *Bishalyakarani*. A certain amount of resemblance exists between this plant and *Ayapana*; but *Jagat Madan* contains a larger percentage

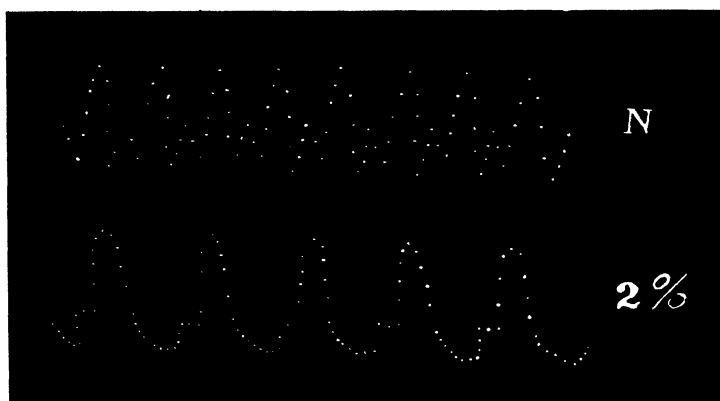


FIG. 129. Effect of extract of *Justicia* (Jagat Madan) on Frog's heart.

First row—normal record.

Second row—effect of 2 per cent. solution.

of nitrates. The juice of this plant is alkaline, while that of *Ayapana* is acidic.

The evaporated *aqueous* extract is a black pasty mass, sweetish in taste and aromatic in odour. A 2 per cent. solution was made with normal saline and applied directly on the frog's heart.

Experiment 160. *Effect of dilute solution of Justicia.*—After taking the normal record, a 2 per cent. solution of *Justicia* was applied on the heart, with the result that there was an increase in amplitude and a slowing-down of the rate of pulsation (fig. 129).

The following table gives typical results of the extract on the amplitude and frequency of the heart-beat :

TABLE XXVII.—EFFECT OF 2 PER CENT. SOLUTION OF
JUSTICIA ON FROG'S HEART.

Applied solution	Magnified amplitude of pulsation in mm.	Frequency of pulsation
Normal . 2 per cent. sol.	12.5 mm. 14.5 "	60 per minute 36 " "

Justicia thus increases the force of the beat and lowers the rate of pulsation.

III. BANAFSHA

This is the Indian name for *Viola odorata*, belonging to the natural order *Violaceae*.

Habitat.—Kashmir ; is also grown in many parts of India.

Description.—A small herbaceous plant, stem short and slender ; leaves caudate and tufted ; root slender.

Uses.—The flowers and leaves are used in the Indian System of Medicine as a diuretic, expectorant, and purgative. It is also used as an antiperiodic and diaphoretic.

Chemical character.—All parts of the plants, specially the roots, contain an alkaloid named *violin*, which resembles emetine. The flowers and stems also contain a glucoside *viola-quiercitrin*, some volatile oil, and sugar.

In the following investigation the extract was made from specimens containing leaves, shoots, and roots, obtained from the market. The prepared extract is a black pasty mass, with a slightly bitter taste and aromatic odour. Three solutions were made from the extract : (I) a 2 per cent.

solution ; (2) a 6 per cent. solution ; and (3) a 10 per cent. solution. The induced physiological change was found from the characteristic variation in the series of records, the first of which may be regarded as typically normal. The succeeding records were obtained with similar specimens but under increasing strengths of dose.

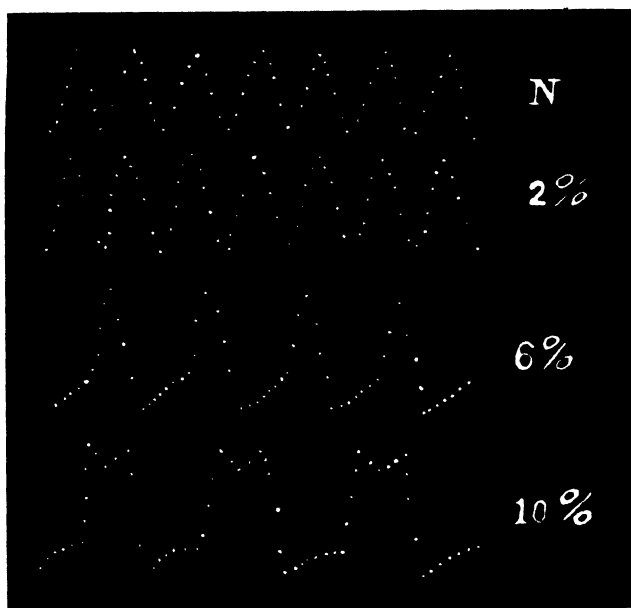


FIG. 130. Effect of extract of *Viola* (Banafsha).
The successive rows of record exhibit the normal activity and variations induced by application of 2 per cent., 6 per cent., and 10 per cent. solutions.

Experiment 161. *Effect of dilute dose of Viola.*—The first row of the record shows the normal activity. On application of a 2 per cent. solution on the heart there is a definite increase in the amplitude of pulsation, the frequency remaining practically the same (fig. 130). The experiment was repeated with a dozen different specimens with precisely similar results.

Experiment 162. *Effect of a stronger dose.*—On the application of a 6 per cent. solution of the extract there was

a marked increase in the amplitude, and a considerable diminution in the frequency of pulsation (*cf.* fig. 130).

Experiment 163. *Effect of a very strong dose.*—In the next series of experiments a 10 per cent. solution was applied over the heart. In this case the increase in amplitude and diminution in frequency is still more marked. The same effect was observed in about a dozen different specimens (*cf.* fig. 130).

The following table gives results obtained with 6 typical cases :

TABLE XXVIII.—EFFECT OF DIFFERENT STRENGTHS OF DOSE OF EXTRACT FROM *VIOLA* ON FROG'S HEART.

Specimen	Applied solution	Magnified amplitude of pulsation in mm.	Frequency of pulsation
1	Normal	13 mm.	81·8 per minute
	2 per cent. sol. .	15 "	81·8 " "
2	Normal	10 mm.	90 per minute
	2 per cent. sol. .	12·5 "	90 " "
3	Normal	10·5 mm.	90 per minute
	6 per cent. sol. .	15 "	56·2 " "
4	Normal	12 mm.	81·8 per minute
	10 per cent. sol.	15 "	64·2 " "
5	Normal	12 mm.	81·8 per minute
	10 per cent. sol.	16 "	64·2 " "
6	Normal	10 mm.	90 per minute
	10 per cent. sol.	17 "	45 " "

The results given above prove that a dilute dose of the

extract enhances the amplitude of pulsation, the frequency remaining the same. Further increase in strength of the dose through a wide range from 6 per cent. to 10 per cent. causes an increase of the force of the heart-beat, while the frequency of the pulsation undergoes a diminution.

IV. HATTISURA

This is botanically known as *Heliotropium Indicum*, and belongs to the natural order *Boraginæ*.

Habitat.—Cochin China ; also found all over India in ditches and in moist places in general.

Description.—A hairy annual, $\frac{1}{2}$ to 2 feet high ; woody branches clothed with soft hairs ; leaves alternate, 1 to 4 inches long and hairy ; flowers pale violet, arranged in spikes 2 to 6 inches long.

Uses.—Leaves are used externally on wounds and ulcers for anodyne action. The investigations described below prove that it has a definite action on the heart.

The extract, prepared from the whole plant in the usual manner, is jelly-like in consistency ; it is black in colour and has a bitter taste. Three different solutions were made : (1) a dilute, 2 per cent. solution ; (2) a 6 per cent. ; and (3) a strong, 10 per cent. solution. I also made etherial and alcoholic extracts from the plants and observed their effects when diluted with water.

Experiment 164. *Effect of moderately strong solution*.—A very dilute solution, such as 2 per cent., had but little effect on the heart. But a 6 per cent. solution induced a marked change in the activity. After application for 5 minutes the amplitude of pulsation was enhanced, while the frequency of pulsation was diminished (fig. 131).

Experiment 165. *Effect of application of a very strong, 10 per cent. solution*.—The application of a strong dose induced not only a depression of amplitude of pulsation, but also a marked slowing-down of the rate of pulsation (see third row, fig. 131).

The etherial and alcoholic extracts, when diluted with



FIG. 131. Effect of extract of *Heliotropium* (Hattisura).
The successive rows exhibit the normal activity and the effects
of 6 per cent. and 10 per cent. solutions.

water, gave results which were similar to those obtained with aqueous solution. The typical results are given below in a tabular form.

TABLE XXIX.—EFFECT OF EXTRACTS OF *HELIOTROPIUM* ON
FROG'S HEART.

Specimen	Applied solution	Magnified amplitude of pulsation in mm.	Frequency of pulsation
1	Normal	10 mm.	90 per minute
	6 per cent. sol.	12.5 "	64 " "
2	Normal	10 mm.	90 per minute
	10 per cent. sol.	4 "	60 " "

The results obtained show that the moderately strong solution, such as 6 per cent., induces a marked increase in amplitude of pulsation, attended by a slowing-down of the rate. The force of the heart-beat is thus considerably

increased. Very strong, 10 per cent. solution induces, on the other hand, a great depression of activity, shown by a reduction of amplitude and a lowering in the frequency of pulsation.

V. GOKSHURI

This is the Sanskrit name of the plant *Tribulus terrestris*, belonging to the natural order *Zygophylaeae*. Its Bengali name is *Gokhru*.

Habitat.—It is found throughout India, specially in sandy soils.

Description.—The fruits consist usually of 5 woody cocci, each with 2 pairs of stiff, sharp spines, forming spiny balls. Fruits and leaves are utilised for obtaining the extract.

Chemical composition.—The extracts of powdered fruit contain alkaloid, resin, fat, and mineral matters.

Uses.—The leaves and fruits are demulcent and are said to have diuretic, tonic, and aphrodisiac properties. They are

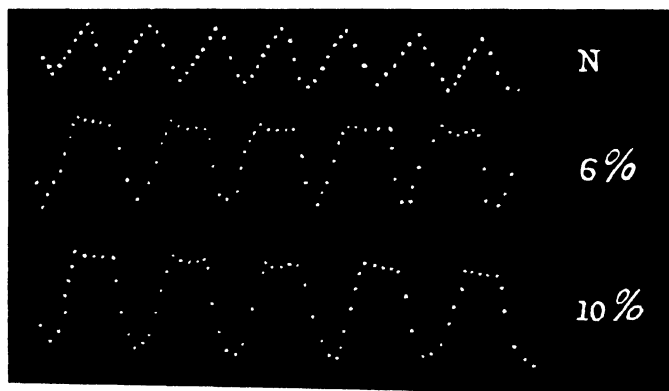


FIG. 132. Effect of *Tribulus* (Gokshuri) on Frog's heart. First row gives normal record. Second and third rows exhibit effects of 6 per cent. and 10 per cent. solutions.

used extensively by followers of the ancient Hindu System of Medicine in diseases of the genito-urinary tract. The stimu-

lating effect of the extract on the heart has been found from the investigations which will be described presently.

Preparation of extract.—The dried fruits obtained from Indian drug stores were crushed in a mortar, and the extract made in the usual manner. The resulting substance was a

TABLE XXX.—EFFECT OF *TRIBULUS* ON FROG'S HEART.

Specimen	Applied solution	Magnified amplitude of pulsation in mm.	Frequency of pulsation
1	Normal	14 mm.	64 per minute
	2 per cent. sol. .	14.5 "	60 " "
2	Normal	6.5 mm.	90 per minute
	6 per cent. sol. .	10 "	64 " "
3	Normal	11 mm.	75 per minute
	6 per cent. sol. .	12.5 "	36 " "
4	Normal	9.5 mm.	81.8 per minute
	6 per cent. sol. .	12.6 "	64 " "
5	Normal	14 mm.	64 per minute
	10 per cent. sol.	17 "	42.8 " "
6	Normal	6.5 mm.	90 per minute
	10 per cent. sol.	13 "	56 " "

brownish dry matter, which became somewhat semi-solid on keeping for some days, owing to absorption of moisture. It has a sweetish taste and aromatic odour. Three solutions were made with normal saline : (1) a dilute, 2 per cent. solution ; (2) a 6 per cent. ; and (3) a strong, 10 per cent.

solution. The results obtained by the application of these solutions on frog's heart are given below.

Experiment 166. *Effect of moderately strong solution of Gokshuri.*—The first row of the record shows normal heart-beat. A 2 per cent. solution induced very little change. But a 6 per cent. solution produced a marked change in the reaction, increasing the amplitude of pulsation and decreasing the frequency of the heart-beat (fig. 132).

Experiment 167. *Effect of very strong solution of Gokshuri.*—The next series of experiments was carried out with 10 similar frogs, a 10 per cent. solution being directly applied on the heart. In every case the reaction was still more marked; there was a further increase of amplitude and lowering of the frequency of pulsation (*see* third row, fig. 132).

The typical results obtained are given in tabular form in Table XXX.

The results obtained show that a very dilute solution of *Tribulus* has very little effect on the frog's heart; stronger solutions, however, produce a marked change in enhancing the amplitude, but decreasing the frequency of pulsation.

VI. CHAKULIA

This is the Bengali name of the plant *Uraria lagopoides*, belonging to the natural order *Rubiaceae*.

Habitat.—It is a grass-like plant found in Nepal, as also in many places in Bengal.

Uses.—It is used for its alterative, tonic, and anticatarrhal properties.

Preparation of extract.—The whole plant, dried and extracted in the usual way, is a black pasty mass, having a sweetish taste and an aromatic odour. Three solutions were made: (1) a 2 per cent. solution; (2) a 6 per cent.; and (3) a very strong, 10 per cent. solution. These solutions gave the following reactions of the frog's heart-beat.

Experiment 168. *Effect of moderately strong solution of Uraria.*—While a 2 per cent. solution applied on the heart produced no alterations either in amplitude or frequency of pulsation, a 6 per cent. solution elicited a moderate increase in the amplitude and a slight diminution in the frequency of the heart-beat (fig. 133).

Experiment 169. *Effect of a very strong solution of Uraria.*—On the application of a 10 per cent. solution the



FIG. 133. Effect of *Uraria* (Chakulia) on Frog's heart.

First row—normal record.

Second row—effect of 6 per cent. solution.

Third row—effect of 10 per cent. solution.

amplitude of pulsation was greatly increased. There was also a moderate diminution of frequency. About 10 frogs experimented on gave similar results (*see* third row, fig. 133).

Table XXXI is a summary of typical results given in a tabular form.

The results given in the table prove that *Uraria* in a dilute solution causes no alteration in normal pulsation. But in strong solutions it increases the amplitude, at the same time moderately slowing down the frequency of pulsation of the frog's heart.

TABLE XXXI.—EFFECT OF *URARIA* ON FROG'S HEART

Specimen	Applied solution	Magnified amplitude of pulsation in mm.	Frequency of pulsation
1	Normal . . . t. sol. .	10 mm. 10 "	81·8 per minute 81·8 " "
2	Normal . . . t. sol. .	11·5 mm. 12 "	90 per minute 90 " "
3	Normal . . . t. sol. .	7 mm. 8·5 "	81·8 per minute 70 " "
4	Normal . . . t. sol. .	11 mm. 12 "	75 per minute 64·2 " "
5	Normal . . . nt. sol.	11 mm. 16 "	75 per minute 60 " "
6	Normal . . . 10 per cent. sol.	11·5 mm. 16 "	90 per minute 60 "
7	Normal . . . 10 per cent. sol.	11·5 mm. 15 "	75 per minute 45 " "
8	Normal . . . 10 per cent. sol.	7 mm. 11 "	81·8 per minute 75

BIBLIOGRAPHY

- BOSE : *Life Movements in Plants*.
 BOSE : *Motor Mechanism of Plants*.
 CUSHNY : *Pharmacology*.
 DYMCK : *Pharmacographia Indica*.
 HALE WHITE : *Materia Medica*.
 KIRTIKAR and BASU : *Indian Medicinal Plants*.
 NADKARNI : *The Indian Materia Medica*.

XII.—INVESTIGATIONS ON EFFECT OF CERTAIN INDIAN DRUGS ON FROG'S STOMACH

BY

N. N. DAS, M.B., M.Sc.

THE animal stomach exhibits a rhythmic activity, similar to that of the heart ; for it has been shown that there is a peristaltic mechanism which is essentially similar in the two cases.¹ As in the heart so also in the stomach :

- (1) The pulsating activity is dependent on the supply of oxygen and is arrested by the asphyxiating action of CO₂.
- (2) The frequency of pulsation is modified by variation of temperature. It is slowed down and becomes arrested at a thermometric minimum, while rise of temperature, within limits, increases the frequency of pulsation.

The difference in the two cases is merely a question of relative quickness, for while peristalsis is very rapid in the heart, it is relatively slow in the stomach. The present investigations were undertaken to study the effect of various plant-extracts, mostly indigenous, on the peristaltic activity of the stomach.

The experiments were carried out with the stomach of the frog, in which, it is to be noted, a considerable difference exists between the activity of specimens in winter and in spring. With the prevailing low temperature in winter, the vitality of the isolated stomach, under proper conditions, can be maintained fairly uniform for nearly 24 hours. But in spring and in summer the isolated stomach, even

¹ Bose, *Motor Mechanism of Plants* (1928), p. 291.

under similar favourable conditions, can be kept alive only for about 12 hours.

The problem which demanded special attention was the devising of a new method for recording the normal peristaltic activity of the stomach and its induced variation under the action of various drugs. The peristaltic movement is usually observed by the method of suspension, the recorded movement being due not merely to diametric but also to longitudinal expansions and contractions. This complication is, however, obviated in the Peristaltograph, devised by Sir J. C. Bose, which records only the pure expansion and contraction of a particular section of the stomach, caused by the passage of the peristaltic wave.

THE PERISTALTOGRAPH

The important parts of the apparatus consist of a short and fixed V-shaped vertical piece, and a primary vertical

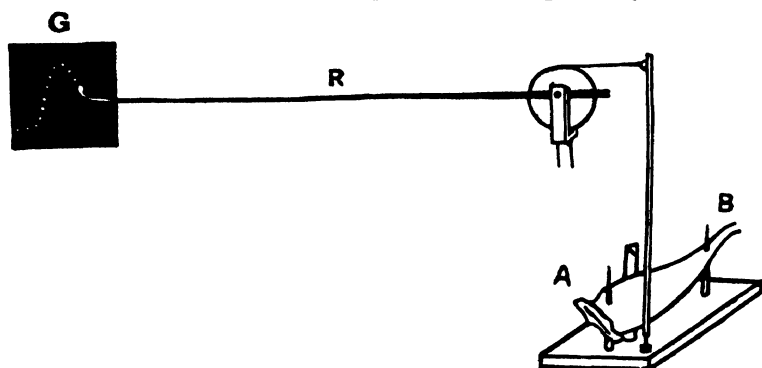


FIG. 134. The Peristaltograph.

Stomach fixed by two pins, the one at the upper or oesophageal end A, and the other at the lower or pyloric end B.

- The middle of the stomach is adjusted between a short and fixed V-shaped vertical piece and a movable long vertical lever. The latter is mounted on a short piece of spring, so that it moves inwards or outwards on contraction or expansion of stomach. The upper end of rod is attached to a small wheel; R, recording lever; G, smoked-glass plate.

lever which is movable. The specimen of the stomach is placed between the two and kept slightly stretched by means

of two pins. The passage of the peristaltic wave moves the primary lever inwards or outwards. This slight movement is further magnified by a wheel of small diameter, which is made to rotate in one direction or the other by the diametric contraction and expansion of the stomach. For purpose of record a long writing lever is attached to the rotating wheel (fig. 134).

The writing lever is made to tap successive dots on a moving smoked-glass plate by means of an electro-magnetic device. The lever is hinged, and a short piece of steel wire is attached to it in a particular position in front of a small electro-magnet M; the steel wire, periodically attracted at equal intervals by an intermittent electric current sent through the electro-magnet, causes the recording lever to make a series of dot-marks on the smoked-glass plate. The intermission of the current is produced by a contact-maker C, actuated by clockwork. The current required for working the electro-magnet is extremely feeble. A small dry cell has been found to last for more than a fortnight for the working of the apparatus. The electric cell is enclosed in the box (fig. 135).

The compound magnification produced by the primary and the recording lever is about 150 times. The magnification can, however, be readily increased to 500 times, when it is easy to record pulsations which had hitherto remained undetected.

The peristaltic wave passes, normally speaking, from the oesophageal to the pyloric end of the stomach. But this normal direction can be reversed under certain conditions. The frequency and force of the peristaltic wave has certain characteristics depending on the condition of the stomach. These characteristics are modified by the action of different drugs, the constituent pulsations being rendered either more vigorous or greatly enfeebled. The frequency of pulsation is, moreover, rendered quicker or slower.

The extract is made by macerating the plant in water. It is then filtered free of chlorophyll and slowly evaporated till a highly concentrated solution is obtained; this maintains its properties unchanged for about a fortnight.

The effect of the plant-extract on the stomach of the frog is complicated by various factors. Thus the efficacy of the active principle obtained from the plant is found to depend on environmental conditions, such as habitat, season,

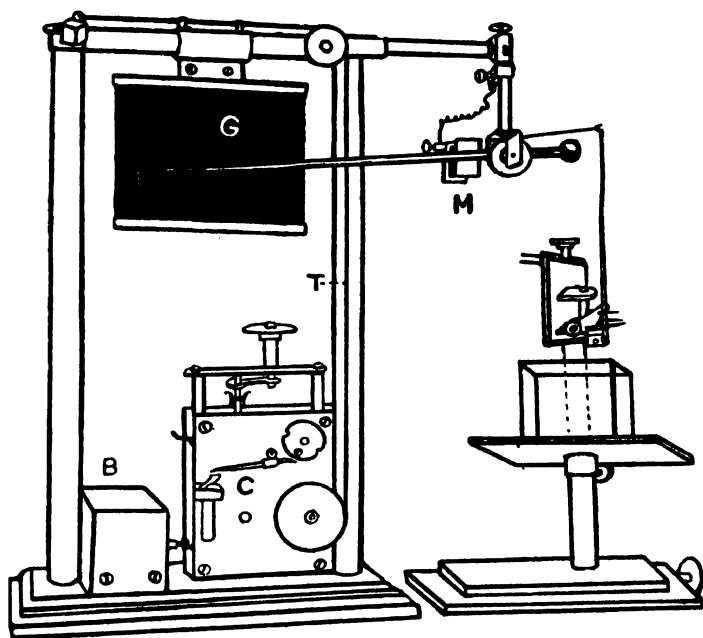


FIG. 135. Recorder for peristaltic movement of Frog's stomach. The peristaltograph seen to the right. The specimen can be placed in a moist chamber, seen below. The steel wire in the hinged recording lever is periodically attracted by electromagnet *M*, and makes dotted record on smoked-glass plate *G*. Intermittent closure of electric current made by toothed wheel. *B*, box containing dry cell. Thread *r*, attached to plate-carrier, is wound round clock-wheel, rotation of which is regulated by governor.

and the character of the soil in which it is grown ; it also depends on whether the plant is grown in a sunny or a shaded place. A further complication arises from the tonicity of the stomach—whether it is in a vigorous or in a subtonic condition.

The investigations were carried out with extracts obtained from the following plants :

- I. *Mirabilis Jalapa*.
- II. *Cassia lanceolata*.
- III. *Trigonella Foenum-graecum*.
- IV. *Ferula Asafoetida*.
- V. *Ipecacuanha*.
- VI. *Plantago Ispaghula*.
- VII. *Asparagus racemosus*.
- VIII. *Malkakni*.

I. MIRABILIS JALAPA

Sanskrit : *Sandhya-raga* or *Krishnakali*

This is a shrub belonging to the natural order *Sapotaceae* of the genus *Nyctagineae*. Though originally imported, it grows extensively in all parts of India, and is generally cultivated in gardens as an ornamental plant. Different parts of the plant, especially its root and leaves, are supposed to contain an alkaloid.

The Extract.—The green leaves from the top of the plant were collected, of which 2 grms. were macerated with saline water to make up 20 c.c. and then filtered. The clear filtrate was used for the investigation.

The isolated stomach of the frog, suitably mounted on the Peristaltograph, was first placed in physiological saline till the irritation caused by section disappeared in the course of about 15 minutes. The specimen was then covered with fine linen, moistened with normal saline, and then lowered into a moist chamber, in which it was kept during the period of experiment. After taking the normal record the plant-extract was applied for observation of the induced effect.

Experiment 170. *Effect of strong solution*.—The writing lever was moving up and down during the passage of peristaltic waves of contraction and relaxation. After starting the lateral movement of the recording plate, the electromagnetic tapper was set in operation and the normal dotted

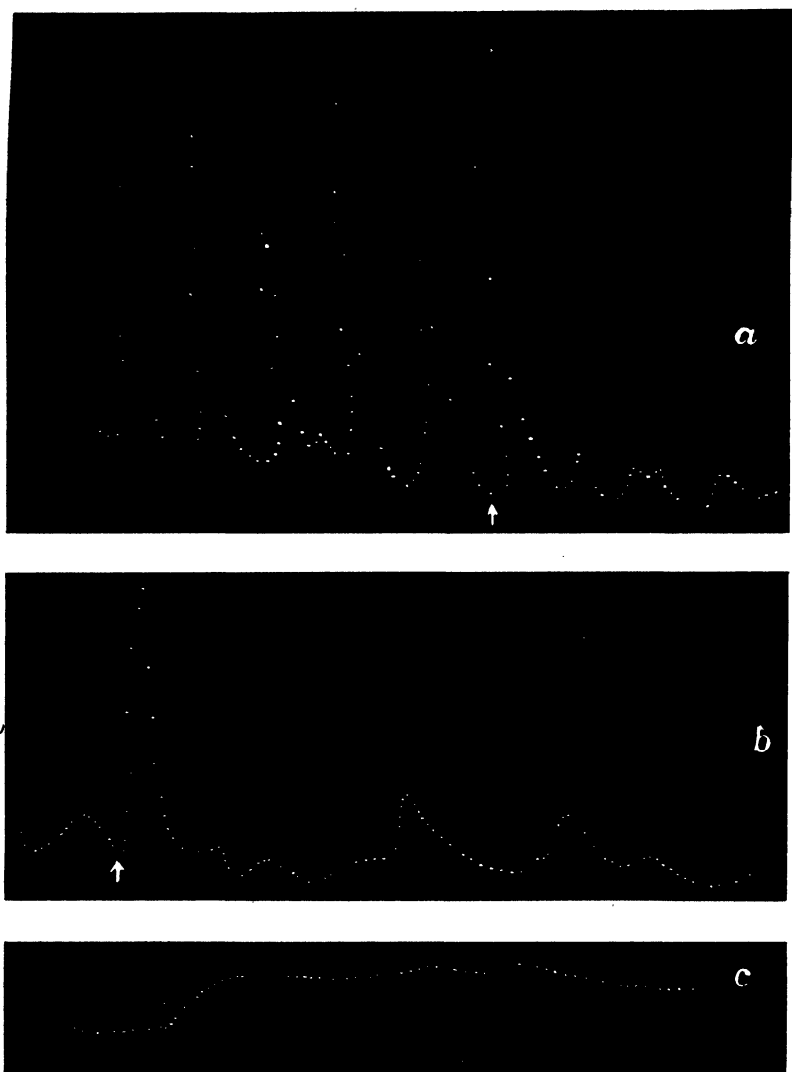


FIG. 136. Effect of strong extract of *Mirabilis* on peristalsis of Frog's stomach.

- (a) Record to left exhibits normal pulsation; application of strong extract at arrow induces transient enhancement of activity followed by depression.
- (b) Further application of extract at arrow causes temporary enhancement and subsequent depression.
- (c) The depression culminates in permanent arrest at contraction in the course of 45 minutes.

curve recorded. The result showed that the contractions were forceful, though somewhat irregular. After application of moderately strong extract of the plant at arrow, there was a short-lived enhancement of activity followed by depression, as seen in the first series of record (a) (fig. 136).

A few minutes after the first application, the solution was applied once more at the next arrow; this resulted in a vigorous contraction followed by depression, as seen in the second series of record (b) in the same figure. Under continued action of the extract the peristaltic wave exhibited increasing depression, and came to a permanent stop at contraction in the course of 45 minutes, as seen in the third series (c) of the record.

A moderately strong solution of the extract thus acts as a poisonous agent, causing an abolition of activity. Further investigations were then undertaken to ascertain the characteristic effects of diluted solutions. The result was found to be very striking, especially when the strength of the solution was so reduced as to be in the proportion of 1 : 500.

Experiment 171. *Effect of dilute solution.*—The normal record obtained with a fresh specimen of frog's stomach is seen in the first series of fig. 137. The activity was moderate and somewhat irregular. The dilute solution, two parts in a thousand, was then applied at intervals; this brought about a definite variation of the character of the pulsations in the course of a few minutes. The pulsations became more forceful and regular, as seen in the second series of record. This characteristic reaction persisted for more than 2 hours, when the pulsations became even more active and regular, as seen in the third series of record.

The results obtained show that a dilute solution acts as a stimulant, causing an enhancement of the amplitude of pulsation as well as a greater regularity; a strong solution, on the other hand, causes a depression and final arrest of activity. Thus the effect of the extract depends on the dose of application.



FIG. 137. Effect of dilute solution of *Mirabilis* on peristalsis of Frog's stomach.

- (a) Normal feeble and irregular pulsation ; dilute solution applied at arrow.
- (b) Effect of dilute extract in inducing greater regularity and increasing the force of contraction.
- (c) Further increase of force of contraction and marked regularity produced in the course of 2 hours.

II. CASSIA LANCEOLATA

(Senna leaves)

This is a well-known plant, largely used for medicinal purposes. It is cultivated almost all over India. The leaves

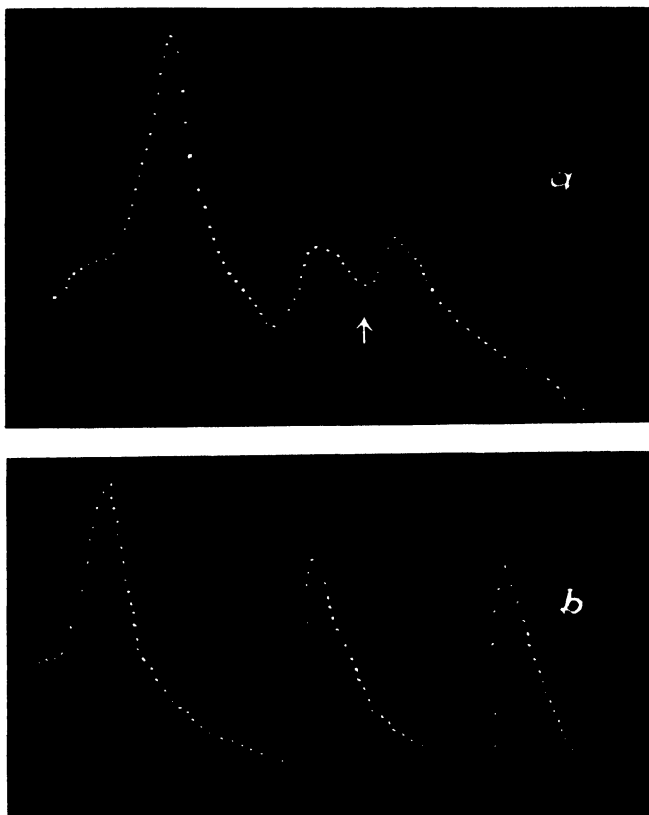


FIG. 138. Effect of dilute solution of *Senna* on Frog's stomach. Irregular pulsation became regular and forceful, the induced change persisting for a long time after application of the extract.

and pods contain various active principles, of which two may be worth mentioning: *Cathartin* and *Sennapicrin*. According to *Cushny* and others, it is one of the anthracene

purgatives, 'the chemical examination of which is a matter of difficulty, as they often contain several active principles which are very nearly related to each other, and some of which are undoubtedly the products of decomposition of more complex bodies.'

The effect of the extract on an isolated stomach has not, as far as I am aware, been studied. The following is an account of experimental investigations which I have been able to carry out on the subject.

The Extract.—Dry leaves of *Senna*, weighing 1 grm., were placed in 20 c.c. of cold water and allowed to soak for 2 hours. For the following experiment 2 c.c. of the solution was diluted with 50 c.c. of physiological saline.

Experiment 172. *Effect of solution of Senna.*—The normal records of peristaltic pulsation of the stomach of the particular frog showed it to be very irregular, as seen in the first record (fig. 138). The dilute solution of *Senna* was now applied on the stomach drop by drop at arrow. The character of the peristaltic activity underwent a gradual change, the pulsations became very regular and of larger amplitude in the course of 10 minutes, as seen in the second series of the record. This enhanced activity persisted for several hours.

The extract of *Senna* leaves thus induces a more regular and forceful peristalsis of the frog's stomach.

III. TRIGONELLA FOENUM-GRÆCUM

Bengali : *Methi*

This is an annual herb, belonging to the natural order of *Leguminosae*, known in Bengali as *Methi*. The plant is cultivated in various parts of Upper India, its seeds being used for condiment. There are various ingredients present in the extract, of which fat and colouring matter are of importance.

The Extract.—One grm. of seeds was macerated with 20 c.c. of physiological saline and afterwards filtered. The filtrate, milky in colour like an emulsion, was employed for the experiment.

Experiment 173. *Effect of extract of Trigonella.*—The

results showed that the effect was greatly modified by the tonic condition of the stomach, a very marked effect being induced on a stomach in a depressed or subtonic condition. This is demonstrated in the following case ; the normal activity of the stomach was very feeble, as seen in the first part of the record ; the application of extract at arrow induced a gradual enhancement of activity, which

FIG. 139. Effect of extract of *Trigonella* on activity of Frog's stomach.

Application of extract at arrow induced gradual enhancement of activity which became persistent, as seen in the lower series of record taken after 40 minutes.

became still more marked and persistent, as shown in the lower series of record taken 40 minutes after the application of the extract (fig. 139).

The extract of *Trigonella* thus enhances the peristaltic activity of the frog's stomach in a depressed or subtonic condition.

IV. ASAFOETIDA

Asafoetida is a substance known from ancient times ; it is a resin exuded from roots of some species of *Ferula*

as a result of incision. The plant, which grows in India and in Persia, belongs to the natural order of *Umbelliferae*. The substance of *Asafoetida* contains several organic compounds of sulphur, to which its strong odour is due. It is used as a condiment. Volatile oil of *Asafoetida* is a carminative and expectorant.

The Extract.—A quarter grm. of *Asafoetida* was mixed in a mortar with 50 c.c. of normal saline ; the solution was milky in appearance.

Experiment 174. *Effect of Asafoetida.*—The stomach of the frog was in a highly irritable condition, as seen in the

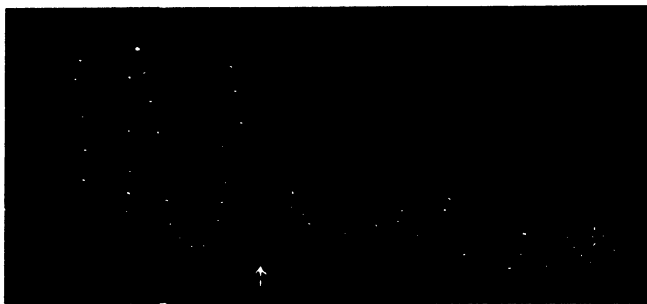


FIG. 140. Effect of *Asafoetida* on Frog's stomach.
First part of record shows normal activity ; application of solution at arrow induced lowering of irritability.

first part of the record. On application of solution of *Asafoetida* at arrow, the peristaltic activity of the stomach became lowered, as seen in the subsequent portion of the record (fig. 140). *Asafoetida* has thus a soothing influence on an irritable stomach.

V. IPECACUANHA

The principal active substance extracted from the root is *Emetine*, so named on account of its action as an emetic.

The Extract.—For this *Vinum Ipecac* was employed, 15 minims being diluted with 10 c.c. of physiological saline.

Experiment 175. *Effect of Ipecac solution.*—After taking a normal record, seen in the first part of the upper record,

the solution was applied on the prepared stomach at arrow. This induced a temporary enhancement of movement. But after a short time the induced increase of activity underwent a change, as seen in the lower series of record, in which the peristaltic activity is seen to have undergone a depression, a condition which persisted for a long time (fig. 141).

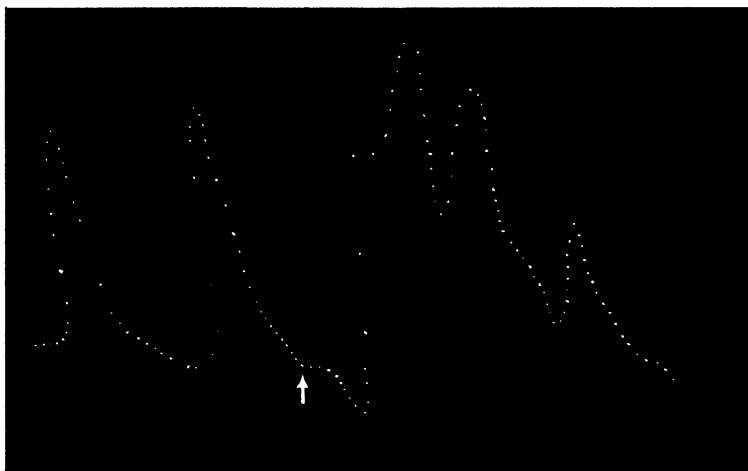


FIG. 141. Effect of solution of *Ipecac* on Frog's stomach. First part of upper record shows normal high peristaltic irritability; there was a temporary enhancement after application of solution at arrow. Continued action of solution induced a lowering of activity, as seen in the lower record.

VI. PLANTAGO ISPAGHULA

The Bengali name of this plant is *Ispaghula*. The seeds when soaked in water become mucilaginous, in which

condition the substance is used by followers of the ancient Hindu system of medicine for the cure of dysentery and other allied disorders.

The Extract.—A quantity of seeds, weighing 1 grm., was crushed in a mortar and soaked in 10 c.c. of physiological saline for half an hour. The mucilage was filtered through

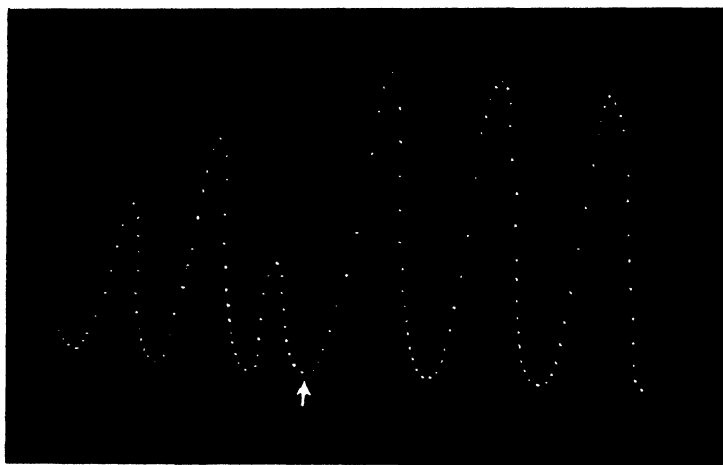


FIG. 142. Effect of solution of *Ispaghula*.

Application at arrow induced a greater regularity and enhancement of amplitude of pulsation, the frequency being decreased.

muslin and the effect of its application on the frog's stomach was studied.

Experiment 176. Effect of Ispaghula.—The first part of the record, seen to the left, shows that the peristaltic activity of the stomach was very irregular. But after the application of the solution at arrow the irregular pulsations became very regular; the amplitude was increased though the frequency was slowed down (fig. 142).

THE METHOD OF PERFUSION

The effect of brief application of the drug on the stomach usually lasts for a short time. It is, generally speaking,

more persistent when the stomach is perfused with the solution.

The Method.—After isolation of the stomach in the usual way, the two cut ends of the stomach are tied to two glass tubes, one at the oesophageal and the other at the pyloric end. Of these the first can be alternately connected with one or the other of two reservoirs by means of a T-tube provided with suitable stop-cocks. One of the reservoirs

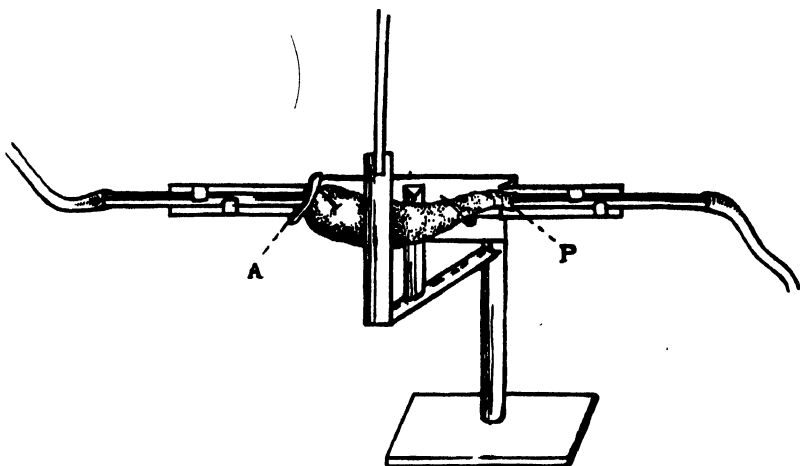


FIG. 143. The method of Perfusion.
A, the oesophageal, and P, the pyloric end of stomach. The inlet tube is attached to the former, and the outlet tube to the latter.

contains the normal Ringer Tyrode solution, while the other contains the drug in proper dilution. The pyloric end of the stomach is tied to an exit glass tube (fig. 143).

The stomach is mounted in the usual manner between the fixed vertical piece and the long movable lever. After the passage of the neutral Ringer Tyrode solution through the stomach for a certain length of time, the normal records are first taken. The solution containing the drug is then introduced by the method of perfusion, and the effect induced by the drug is then recorded.

VII. ASPARAGUS RACEMOSUS

This climbing plant is found all over India, and is known in Bengal as *Shatavari*. It belongs to the natural order *Liliaceae*; the roots are used both as medicine and food.

The Extract.—Dried roots weighing 2 grms. were crushed in a mortar and soaked in 20 c.c. of saline and filtered after half an hour. The filtrate, thoroughly mixed with 250 c.c. of Ringer Tyrode solution, was poured into the second reservoir for the purpose of perfusion.



FIG. 144. Effect of solution of *Asparagus*.

The first part of the record shows the normal activity; the second part after arrow shows the effect of the drug in enhancement of activity.

Experiment 177. *Effect of solution of Asparagus.*—Normal record is taken during the passage of the neutral Ringer Tyrode solution through the stomach. The solution of the drug is then perfused through it, and its effect recorded. In the first part of fig. 144 is shown the normal record; the second part exhibits the great enhancement of peristaltic activity induced by the drug on the stomach.

VIII. MALKAKNI

The above is the Bengali name of the plant, the seeds of which are used for medicinal purposes. They are deep

yellowish-red in colour, and have a pungent smell. When macerated with water the solution becomes canary-yellow in colour.

The Extract.—Five grms. of the seed are macerated with 50 c.c. of water. The fluid mixed with normal saline is made to perfuse through the stomach.

Experiment 178. *Effect of Malkakni solution.*—The normal record is taken at the beginning, and the subsequent



FIG. 145. Effect of solution of *Malkakni*.

The first part of the record shows the normal activity of the stomach. The second part shows the effect of the drug.

record exhibits the effect of the drug. The activity of the particular specimen was feeble and irregular, and the result of application of the drug was found to cause a considerable enhancement of the peristaltic activity, which persisted for a long time (fig. 145).

BIBLIOGRAPHY

- BAYLISS : *Principles of General Physiology*.
 BOSE : *Motor Mechanism of Plants*.
 CUSHNY : *Pharmacology and Therapeutics of the Action of Drugs*.
 NADKARNI : *The Indian Materia Medica*.
 PITTENGER : *Biologic Assays*.

XIII.—THE FISH-EATING SPIDERS IN BENGAL AND THEIR HABITS

BY

G. C. BHATTACHARJEE, VIDYARATNA

THE study of spiders which capture and eat fish and tadpoles has engaged the attention of investigators all over the world. The American Society of Natural History have from time to time deputed workers to make a collection of them and to make a survey of their natural habitat. Pickard Cambridge refers to the fish-eating spiders of the genera *Dolomedes* and *Thalassius* as follows :

‘ The spiders belonging to the *Pisauridae* are interesting from the fact that they run freely and with great rapidity over the surface of the water, even in a strong current. One of the genera, *Dolomedes*, and another, *Thalassius*, have both been credited with capturing and devouring small fish of various kinds, and of the latter genus, Mr. A. N. Stenning, himself formerly a gamekeeper, whose observations are likely to be trustworthy, declares that he has found them actually devouring the small fry of a variety of trout which occurs in South Africa, some of the culprits being now in the museum collection.’¹

He further refers to the genus *Trachalea* :

‘ The habits of these spiders are very interesting, for although they are not amphibians in the true sense of the term, as are *Argyroneta desis*, they are quite at home on water and speed along over the surface with great rapidity. I have never observed them dive below the surface as do *Pirata*, *Dolomedes*, and other *Pisauridae*. These spiders are

¹ F. Pickard Cambridge, ‘ On New Spiders,’ *Proc. Zool. Soc. London*, Feb. 17, 1903, vol. i, p. 152.

reported to prey upon fish, though I cannot say this from actual experience.' ¹

Dr. H. McCook has referred to a fish-eating spider which is regarded by him as an example of *Lycosa lenta* or of *Lycosa fatifera*, or more probably of *Dolomedes tenebrosus*, and which is said to grow to a considerable size.² Dr. McCook makes further reference to Mr. Francis R. Welsh's observation of a fish-eating spider which is supposed to be either a *Dolomedes* or *Agalena navia*.³ A very different type of fish-eating spider is described by Mr. C. H. Robson.⁴ Messrs. D. R. Crawford and C. N. Breder have written about certain fish-eating spiders, the accounts which they gave being, however, not sufficient for their identification.⁵ Mr. Wallace Adams of Steinhart Aquarium in San Francisco also describes his observations on certain fish-eating spiders.⁶ Professor J. H. Comstock has given an account of a fish-eating spider which, according to him, possesses the habits of *Dolomedes tenebrosus*.⁷ Dr. Thomas Barbour, of the Museum of Comparative Zoology, Harvard University, has written of a fish-eating spider belonging to the genus *Dolomedes*.⁸ Mr. William T. Davis refers to a fish-eating spider which belongs to *Dolomedes sexpunctatus*.⁹ Mr. T. M. Peters has given an account of a species of a fish-eating spider; he was, however, not sure whether it was a *Lycosa* or a *Dolomedes*.¹⁰ There is an account of Mr. Abraham's observation of a fish-eating spider published in 'Nature' by E. C. Chubb,¹¹ which has been identified as *Thalassius spenceri*.

¹ F. Pickard Cambridge, 'On New Spiders,' *Proc. Zool. Soc. London*, Feb. 17, 1903, vol. i, p. 158.

² McCook, *American Spiders and Their Spinning Works*, vol. i, p. 273.

³ *Ibid.*, vol. iii, p. 66.

⁴ C. H. Robson, *Trans. and Proc. of the New Zealand Institute*, 1877, vol. x, pp. 200-201.

⁵ Crawford and Breder, quoted by Dr. Gudger in *Nat. Hist. Mag.* (New York), 1925, vol. xxv, No. 3, pp. 261-263.

⁶ W. Adams, *Bull. of New York Zool. Soc.*, 1927, p. 77.

⁷ J. H. Comstock, *Spider Book* (New York), 1912, p. 186.

⁸ Barbour, 'Spiders Feeding on Small Cyprinodonts,' *Psyche*, 1921, vol. xxviii, pp. 131-132.

⁹ William T. Davis, 'A Spider Fisherman,' *Entomological News*, April 1891, vol. ii, p. 77.

¹⁰ T. M. Peters, *The American Naturalist*, 1876, vol. x, p. 688.

¹¹ E. C. Chubb, *Nature*, 1913, vol. xci, p. 136.

Nendick Abraham of Pietermaritzburg, Natal, refers in the 'Annals of the Natal Museum' to various instances of fish killed by spiders. From these facts he came to the conclusion that :

'A fish-eating habit has been established for the species of *Dolomedes* in America and for the species of *Thalassius* in South Africa. These genera belong to the family *Pisauridae*, and are closely allied. The tadpole-eating habit has been observed in *Diapontia* of the family *Lycosidae* in America and in a species of *Thalassius* in South Africa. Thus the habit of catching tadpoles occurs in two spiders belonging to different families. This fact favours the view that the fish- and frog-eating habit has been acquired independently in different geographical regions.'¹

A very exhaustive account has been contributed by Dr. E. W. Gudger in the 'Natural History Magazine,' New York,² about all fish-eating spiders in different parts of the world ; there is, however, no mention of such spiders in Bengal. This led me to investigate whether they are to be found in this country, and, if so, to make a detailed investigation as to their general habits, their mode of catching fish, as well as various characteristics associated with their mating. Such observations on spiders in a different climate and habitat would undoubtedly prove of general biological interest. My efforts were specially directed towards obtaining instantaneous photographs at most critical moments of their activities, so that errors associated with mere eye observation might be completely eliminated. The fish-eating spider in Bengal, which is described in this Paper, has been kindly identified for me by Dr. Gravely as *Lycosa annandalei*.³

GENERAL OBSERVATIONS ON THE HABITS OF *LYCOSA ANNANDALEI*

Early in March 1931 my attention was attracted to a full-grown spider of greyish colour on the surface of a

¹ Nendick Abraham, 'Observations on Fish- and Frog-Eating Spiders of Natal,' *Annals of Natal Museum* (Pietermaritzburg), 1923, vol. v, pt. i, p. 89.

² E. W. Gudger, *Nat. Hist. Mag.*, 1922, vol. xxii, No. 6 ; 1925, vol. xxv, No. 3 ; 1931, vol. xxxi, No. 1.

³ Gravely, *Records of Indian Museum*, vol. xxvi, pt. vi, pp. 606-607.

stagnant pool in the suburbs of Calcutta. The pool was full of *Hydrilla*, *Limnanthemum nymoides*, and other aquatic plants. This particular creature completely paralysed a spider of a different species by inserting its fangs right through the dorsal thoracic portion of the victim, holding it in that position for a certain length of time till death ensued ; after this the prey was devoured. Subsequently I chased the spider from place to place for a considerable length of time till it became tired ; then it feigned death by folding its legs and floating on its back in an inverted position. When I attempted to pick it up, it suddenly disappeared. This disappearance, I found later, was due to the sudden diving of the spider under water, where it remained for more than fifteen minutes.

Characteristic habits.—The fish-eating spiders are of an amphibious habit. They spend most of their day on water, sometimes floating on it or resting upon leaves of aquatic plants. With the approach of evening they usually retire to land, hiding under vegetation. Sometimes they creep under bricks and pebbles and rest there for the night. Though fond of sunshine, they usually avoid it when it is too strong, as in the middle of the day ; they then take shelter under bushes. In the daytime they hop over wide stretches of water by quick jumps. Sometimes they rest upon the clear surface of water, slight depressions being made on the surface under their feet, without breaking the 'water-skin.' Diving under water is a peculiar characteristic of these spiders. If frightened or chased by enemies they suddenly dive and remain clinging to the aquatic plants. They look silvery white under water, because of the air coat round their bodies, which protects them from getting wet. The mother spider, when in danger, also dives several inches below water with the cocoon or younglings on her back and creeps along aquatic plants ; in this way they can move to a considerable distance and succeed in hiding themselves in places of safety.

Sources of food.—These particular spiders prey upon various insects, especially the waterflies that float upon the surface of the water, and also on dragon-flies. Most of

them are cannibalistic in their habits, preying upon the weaker ones. The female spiders often devour the males. They spin a little, and that only for their cocoons ; *but they do not weave any web, nor do they construct snares for catching their prey.* They capture fish rather by direct attack, of which the following is a detailed account.

METHOD OF CAPTURING FISH

In a pond of water at Dum Dum I found a large number of diving spiders. Numerous sun-fishes or minnows (*Elas-soma zonata*) were also swimming about in the same pond. When frightened the fishes took shelter under floating leaves and remained there until the danger was over. I noticed some four or five such fishes feeding at the edge of a small nymphoid leaf ; a female spider was also seen, sitting at the centre of the leaf and watching the fishes patiently for a long time. From the attitude of the spider, a cursory observer would have concluded that she was absolutely indifferent to the movements of the minnows. The case was, however, very different ; for the spider crept very slowly from the centre towards the edge of the leaf by alternately advancing and then stopping for a while. When sufficiently near she suddenly fell upon one of the small fishes about three-fourths of an inch in length ; she caught the fish by the neck and inserted her poison fangs into it. In vain did the fish struggle to set itself free ; the spider was in a secure position and succeeded in dragging the fish on to the leaf, where after a brief struggle it became completely paralysed, and died subsequently.

PHOTOGRAPHING FISH-CAPTURE

For further observation I collected a large number of these spiders and placed them in a glass reservoir with a small quantity of water, in which were also placed some aquatic plants and half a dozen minnows. One of the minnows was missing on the third day, and the number

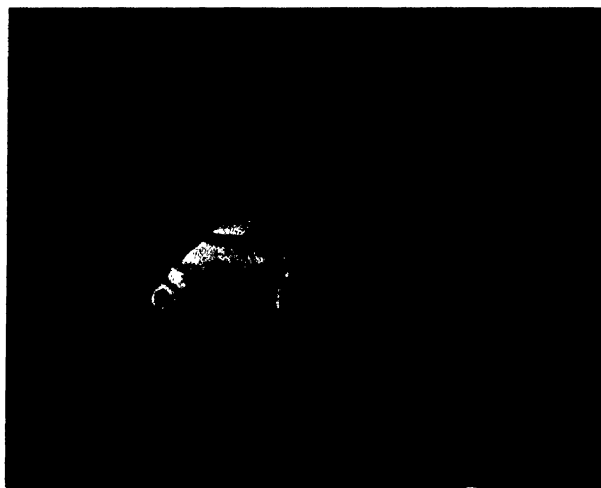
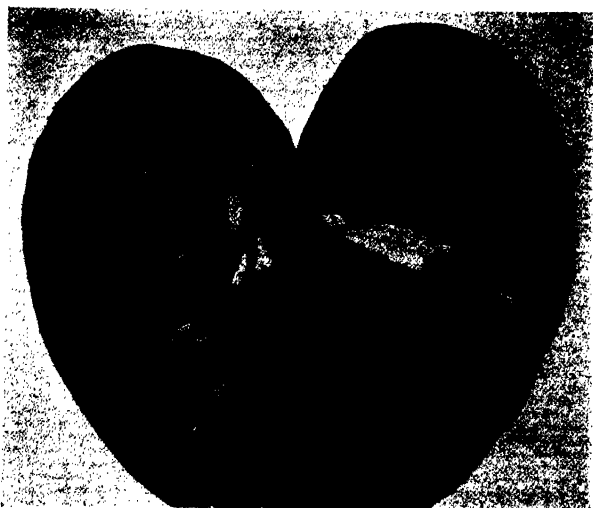


FIG. 146. Photograph of capture of fish by water-spider (magnification 1.5 times).
Upper illustration.—Fish dragged on to the leaf by spider, which, disturbed by loud sound, releases it.
Lower illustration.—Process of devouring the fish (leaf not seen).

gradually decreased till on the tenth day all had disappeared. Evidently they had been devoured by the spiders.

I next attempted to photograph these spiders in the very act of capturing and devouring the fish. The task proved to be exceedingly difficult. Success, however, attended my efforts after I had kept the spiders in a shallow vessel containing water for five days without any food, so that they became extremely hungry. After the spiders had

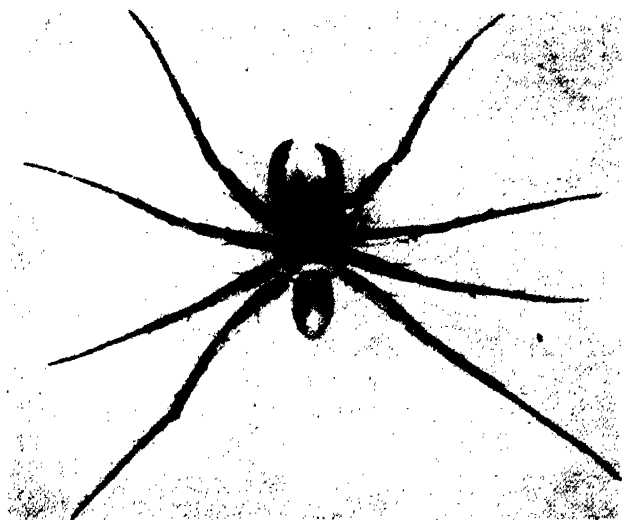


FIG. 147. Photograph of the male spider
(magnification 2 times).

become accustomed to their new surroundings, a number of minnows were introduced into the vessel, in which they swam about vigorously. One of the spiders now caught hold of a fish, and I took this favourable opportunity to photograph the spider in the process of capturing its prey.

In the upper of the two illustrations (fig. 146) the fish captured by the spider and dragged on to the leaf is seen released, on account of the spider having been frightened by a loud sound which was purposely made. In the lower illustration of the same figure there was no such disturbance, and the spider succeeded in pulling the fish on to the

leaf and in completely paralysing and killing it by its poison fangs. After having done this the spider greedily devoured the fish. In order to have a clear view of the process, the leaf on to which the fish has been dragged is omitted from the illustration.

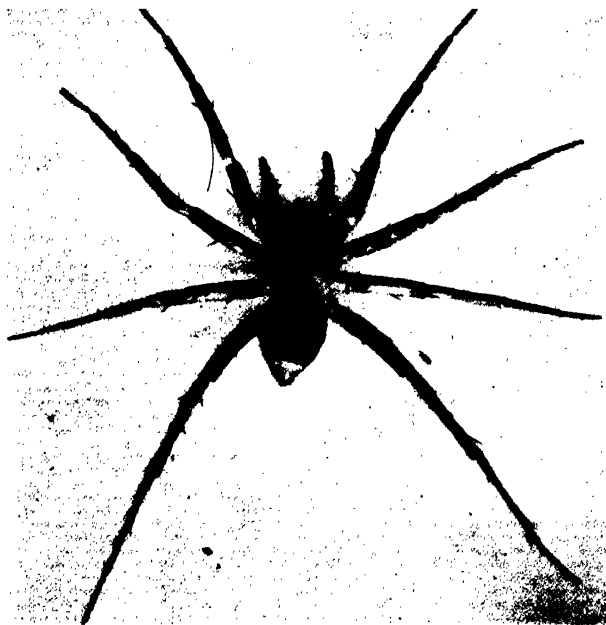


FIG. 148. Photograph of the female spider
(magnification 2 times).

When the vessel containing the starving spiders was supplied with tadpoles, the latter were attacked and paralysed by the poison fangs. The spiders, however, did not appear to relish the tadpoles as much as they did the minnows.

THE MATING OF FISH-EATING SPIDERS

The following account of courting and mating of the water spiders may be of interest. The general habits of both males and females appear to be somewhat similar ;

they move about in the same places, though the males keep at a safe distance from the females, being afraid of a sudden attack. The males are smaller in size (*cf.* figs. 147, 148) but are more agile and swift in their movements. During the breeding season I was fortunate enough to see the process of courtship, as the male approached the female by a succession of peculiar dancing steps. The advance of the male is very slow, as if he were counting the steps and at the same time raising and lowering the body alternately. The male thus approaches the female, the dancing movement going on sometimes for several hours. All the while the female sits quietly, intent on watching the male. At too near approach of the male, the female frightens him by raising her forelegs and thus driving him to a distance. The male then keeps quiet for a few minutes, after which he again approaches her, vibrating the legs and dancing vigorously. This procedure is repeated many times, the female all the while remaining apparently passive. Then all of a sudden the male jumps on her back, clasping her tightly round the cephalothorax with two pairs of his middle legs, the female, for the time being, becoming quite docile.

FERTILISATION OF THE FEMALE SPIDER

The process of fertilisation of spiders is generally supposed to be an indirect one. The testes of the male spider lie in the abdomen, and it is thought that before mating the male deposits a drop of sperm on a sheet of webbing. The sperm is subsequently reabsorbed into the palpal organs, which are applied during mating to the epigynum underneath the abdomen of the female. The question next arises : How could the above process be in operation in the case of the fish-eating spiders, which do not weave any web, and therefore the male cannot deposit the sperm on it for subsequent reabsorption into his palpal organs ? Since this is impossible under the particular circumstances of the case, could the introduction of the sperm into the female organ of the fish-eating spiders prove to be a direct process ?

After drawing attention to the apparent anomaly of the

subject, I shall content myself with describing my observations in regard to the copulatory process of the fish-eating spiders, illustrating the fact with an instantaneous photo-



FIG. 149. Mating of *Lycosa annandalei*.

The upper spider is the male. The female is under the male. The abdomen of the female has been turned sideways by the male. The palpal organs *p* are seen upon the epigynum of the female (magnification 2 times).

graph which I have been very fortunate to secure. The male, by the help of his front legs, was seen to turn the abdomen of the female sideways within easy reach of his palpal organ, which was thrust to the utmost into the

genital organ of the female (fig. 149), the sperm being injected about five times, each after an interval of four seconds. This is what occurred on one side of the female, which was next turned over to the other side and the process repeated once more for about 20 seconds. The alternate turning of the abdomen and insertion of the male organ went on for more than 15 minutes.

While the process of mating was in progress I was able to capture both the male and the female and place them in a wide glass cylinder, through the walls of which I could observe subsequent events. When the mating was over the male suddenly released the female and then tried to escape out of the cylinder ; being unsuccessful in this attempt, he retraced a few paces backwards. The female now rushed at him with great fury, and, catching him by the neck, pierced his cephalothorax with her fangs. This caused the death of the male within a few minutes. The female was relentless in her ferocity, for a similar fatal end awaited a second male spider which was thrown into the cylinder.

The female, thus fertilised, was kept in captivity ; after sixteen days she laid her eggs. It is a curious fact that spiders kept under observation in captivity laid their eggs at night. These eggs, enclosed in a thin, pea-like cocoon, were subsequently carried along by the mother spider till they were hatched in the course of about a fortnight. The number of young spiders which came out of a single cocoon often exceeded 150. The young ones thus hatched climb on the back of their mother in a pile and are thus carried about from place to place.

The cocoon is carried by the mother by means of the spinnerets. When the cocoon is forcibly taken away from her and then thrown to a distance, she picks it up again. When a cocoon was stuck to a lump of paraffin, the mother tried her utmost to detach it. The mother spider, seemingly so attached to the cocoon, is, however, unable to distinguish her own from those belonging to others. When a substitution was made of the cocoon, the mother was quite satisfied with the substitute. When several different cocoons, of nearly equal size, were mixed up, the mother was unable

to differentiate between them and carried away any one out of the lot at random. In regard to the newly hatched spiders, they may be transferred from the back of one mother spider to that of another, without any notice being taken of the exchange.

I have in this Paper described the habits and mating characteristics of the fish-eating spider *Lycosa annandalei*, which is found in Bengal. In regard to internal anatomical structure, there is nothing which requires special mention. I further studied external characteristics such as markings, number and arrangement of eyes, the number of teeth in the mandible, for purposes of identification; these are, however, not of sufficient importance to be described here.

In conclusion, I take this opportunity of expressing my grateful thanks to Sir J. C. Bose, who has encouraged me in this work in every possible way. To Dr. F. H. Gravely I am indebted for the identification of the spiders sent to him. My best acknowledgments are also due to the helpful suggestions received from Dr. B. K. Das, Dr. K. N. Bahl, Mr. J. P. Sircar, and Professor N. C. Nag.

XIV.—THE PROTEOLYTIC ENZYMES OF *CARICA PAPAYA* (III)

BY

N. C. NAG, M.A., F.I.C., AND H. N. BANERJEE, M.Sc.

IN our previous Papers¹ on *Carica Papaya* published in the 'Transactions of the Bose Institute, 1930-31,' certain evidence was produced supporting Professor Vines' conclusion that two types of proteolytic enzymes occur in plants—namely, (1) the peptic and (2) the ereptic, in regard to which we were able to show that unripe papaw fruit juice contained the two types, which were separated by shaking the juice with kaolin and centrifuging the material; of these the sludge carried down the adsorbed peptase, which was brought into solution by eluting the sludge with NaCl solution. The filtrate or the supernatant liquid, on the other hand, contained the ereptase.

The present investigation was undertaken in order to ascertain whether or not the two enzymes could be isolated from commercial papain. Solutions of ordinary papain (clean white samples) obtained from chemists, selling at the rate of 2s. per oz., and Finkler's papain (giving slightly brownish extract) valued at twice or thrice that price, were compared with fresh papaw juice as regards their proteolytic activity on shreds of fibrin.

Experiments were also made with solutions containing HCN of various strengths with addition of Na_2CO_3 , in order to find out at what CN concentration the fibrin was most quickly dissolved. Afterwards we substituted, for HCN and Na_2CO_3 , solution of KCN, which contained equivalent

¹ Nag and Banerjee, *Trans. Bose Institute*, 1930-31, chaps. xviii, xx.

quantities of CN, and made observations as to the best concentration which was most effective. The *pH* values of the different solutions were also determined with a view to finding out the optimum range.

THE PROTEASES OF PREPARED PAPAIN

Finkler's papain was found to have the same properties as papain which was obtained from unripe papaw fruit juice by precipitation with alcohol; that is to say, it dissolved fibrin but did not proceed any further in producing tryptophane. Beyond the fact that the action of papain thus prepared from fresh papaw juice by alcohol precipitation was more rapid, there was no evidence of any essential difference in their character, since both contained peptase only.

COMPARISON OF FINKLER'S PAPAIN WITH COMMERCIAL VARIETY

A.—*Five grms. of Finkler's papain* were stirred with 200 c.c. of *distilled water* for half an hour in a porcelain mortar. The mixture was then centrifuged and a clear, slightly brown supernatant liquid was obtained. This solution to begin with was faintly acid in reaction, and gave weak biuret but no tryptophane indication. This solution was put in three bottles, X, Y, and Z, with the object of finding out whether the solution contained any peptase or ereptase, as also to be sure if there were any autolysis. The detailed description and comparative results are given below.

Bottle X.—40 c.c. of the above solution were made alkaline to the extent of 0.75 per cent. Na_2CO_3 . To this were added 20 drops (1 c.c.) 5 per cent. HCN and then 0.1 gm. moist fibrin shreds.

Bottle Y.—40 c.c. of the solution with 20 drops of 5 per cent. HCN and 0.1 gm. Witte-peptone.

Bottle Z.—40 c.c. of the solution with 20 drops of 5 per cent. HCN for autolysis.

Bottles	November 9			November 10			November 12	
	Trypt	Biuret	Fibrin	Trypt	Biuret	Fibrin	Trypt	Biuret
X, Fibrin	Nil	Faint	Dis-solved	Nil	Dis-tinct	Dis-solved	Nil	Strong
Y, Witte-peptone*	Nil	Strong	—	Nil	Strong	—	Doubt-ful	Strong
Z, Auto-lysis	Biuret reaction all through, but no tryptophane							

ORDINARY COMMERCIAL PAPAIN.

(Observations taken at 7 A.M. each day.)

Bottles	November 9			November 10			November 12	
	Trypt	Biuret	Fibrin	Trypt	Biuret	Fibrin	Trypt	Biuret
X', Fibrin	Nil	Faint	Broken up	Nil	Dis-tinct	Dis-solved	Nil	Strong
Y', Witte-peptone	Faint	Strong	—	Dis-tinct	Strong	—	Dis-tinct	Strong
Z', Auto-lysis	Nil or doubtful	Faint	—	Faint	Faint	—	Faint	Faint

Control experiment (distilled water, Witte-peptone, and HCN) showed biuret reaction throughout but no tryptophane.

From the above it will be seen that the ordinary commercial papain gives evidence of a feeble ereptic and a marked peptic reaction, the rate of fibrin-dissolution being very much slower than in the case of Finkler's papain. Finkler's papain, however, shows only peptic activity. In the ordinary variety, it would appear that the ereptase was not wholly eliminated during its preparation.

We next attempted to find out if there was anything left after extraction with distilled water in the above cases. For this the residue in each case was further treated with 5 per cent. NaCl solution. These were placed in bottles, A and B, for examination of the presence of peptase and ereptase by following the procedure already described.

A.—20 c.c. NaCl extract from Finkler's papain residue were treated with 10 drops 5 per cent. HCN and 0.1 grm. of fibrin added.

B.—20 c.c. NaCl extract obtained from *ordinary commercial papain* residue were taken, to which 10 drops of 5 per cent. HCN were added and 0.1 grm. fibrin introduced.

The experiments were started at 5 P.M. on November 8, 1930, by placing the bottles in the incubator and observations continued as in previous cases in the mornings.

OBSERVATIONS ON FINKLER'S AND ORDINARY PAPAIN RESIDUE
(AFTER DISTILLED WATER EXTRACTION).
(Final extraction with NaCl solution.)

Bottles	November 9			November 10			November 12	
	Trypt	Biuret	Fibrin	Trypt	Biuret	Fibrin	Trypt	Biuret
A	Nil	Strong	Dissolved	Nil	Strong	Dissolved	Doubtful	Strong
B	Nil	Strong	Dissolved	Nil	Strong	Dissolved	Distinct	Strong

Remark.—The reactions are the same in both, only tryptophane was in evidence on the last day in ordinary papain contained in bottle B. The experiments just described were made with extracts in 5 per cent. NaCl solution of the residues left after extraction in distilled water. As will be observed from the time taken in fibrin-dissolution, the peptic enzyme is more soluble in NaCl solution than in distilled water. As has been remarked, faint tryptophane reaction was in evidence on the morning of November 12. During the first two days of observation with fibrin, there was no evidence of tryptophane. This would point to the presence of ereptase in extremely small quantity in Finkler's papain, if any at all; it is present in slightly larger quantity in the ordinary variety. Certain samples of Finkler's papain seemed to be more free from ereptase than others. Both seem to be similar to the product obtained by precipitation with alcohol.

An additional series of observations was taken by first incubating the bottles of extracts before introduction of the fibrin. As a result of this preliminary raising of the temperature, the time taken in fibrin-dissolution became very much shortened.

EFFECT OF PREVIOUS INCUBATION

Two bottles were placed in the incubator at 40° C. on November 11 at 7.45 A.M. Of these—

Bottle 1 contained 1 grm. of Finkler's papain in 40 c.c.
5 per cent. NaCl solution with 20 drops HCN; and

Bottle 2 contained 1 grm. of ordinary papain in 40 c.c.
5 per cent. NaCl solution with 20 drops 5 per cent.
HCN.

Observation.—By 9 A.M. of the same day the bottles had attained the temperature of the incubator, *i.e.* 40° C. They were now taken out and to each of the bottles was added Na₂CO₃ to the extent of 0.75 per cent. and one shred of fibrin. The shreds of fibrin became quickly dissolved even before the bottles were once more replaced in the incubator by 9.30 A.M., when additional pieces of fibrin were put in. These additional shreds of fibrin became dissolved in the course of half an hour.

On the morning of the next day, *i.e.* on November 12, the solutions in the bottles 1 and 2 were subjected to further test; they gave distinct biuret reaction, but there was no indication whatsoever of the presence of tryptophane.

Similar experiments were performed with papain prepared in the Bose Institute laboratory, with exactly similar results, showing that the time for the dissolution of fibrin is not only quickened in an adequately alkaline solution (*pH* value about 8.5), but is further shortened if the enzyme solution, after addition of alkali cyanide, be previously raised in temperature in the incubator. The following is an instance of the effect of this preliminary incubation of a specimen obtained from unripe fruit juice. The following experiment was carried out on November 16:

50 c.c. of expressed juice from unripe papaw fruit pulp with 25 drops of 5 per cent. HCN and 0.75 per cent. Na₂CO₃ were placed in the incubator for about half an hour. When 0.1 grm. of fibrin was added it became completely dissolved in about 15 minutes. Fresh pieces also were dissolved in about 20 minutes.

EFFECT OF CN CONCENTRATION ON DISSOLUTION

For the following experiments, expressed juice from unripe papaw fruit from the Bose Institute Gardens was used on November 21.

Bottle 1 contained 40 c.c. of juice with 1 c.c. of 2 per cent. HCN.

Bottle 2 contained 40 c.c. of juice with 2 c.c. of 2 per cent. HCN.

Bottle 3 contained 40 c.c. of juice with 4 c.c. of 2 per cent. HCN.

Bottle 4 contained 40 c.c. of juice *without any* HCN.

They were all placed side by side in the incubator for half an hour for preliminary raising of the temperature. After this, into each of the bottles was put 0.1 grm. fibrin. The bottles were then replaced in the incubator at 2 P.M. At 2.30 P.M., when the first observation was taken, the fibrin in none of the bottles had been attacked. This only showed that fibrin dissolution did not take place quickly in an acid medium where pH value was below 7.

At 2.30 P.M. the bottles were made alkaline as usual to 0.75 per cent. Na_2CO_3 and replaced in the incubator. At 3.30 P.M. there was complete dissolution of the fibrin in bottles 2 and 3, in which the quantity of HCN was considerable (2 c.c. and 4 c.c.), while fibrin in bottles 1 and 4 (containing 1 c.c. HCN and none at all) was merely broken up.

The next series of experiments was carried out several months later, on April 1, 1931. In these, clear centrifuged juice from unripe papaw fruit was made alkaline at the very beginning; to these were added increasing quantities of HCN as detailed below.

Bottle 1 contained 40 c.c. of juice with 1 c.c. of 2 per cent. HCN and 0.75 per cent. Na_2CO_3 .

Bottle 2 contained 40 c.c. of juice with 2 c.c. of 2 per cent. HCN and 0.75 per cent. Na_2CO_3 .

Bottle 3 contained 40 c.c. of juice with 4 c.c. of 2 per cent. HCN and 0.75 per cent. Na_2CO_3 .

Bottle 4 contained 40 c.c. of juice with 8 c.c. of 2 per cent. HCN and 0.75 per cent. Na_2CO_3 .

Bottle 5 contained 40 c.c. of juice with 10 c.c. of 2 per cent. HCN and 0.75 per cent. Na_2CO_3 .

Bottle 6 contained 40 c.c. of juice with 0.75 per cent. Na_2CO_3 and no HCN.

All the bottles were placed in the incubator at 12.30 P.M. After half an hour, *i.e.* at 1 P.M., fibrin was introduced into each one of the bottles, which were replaced in the incubator and observations were taken at intervals of 15 minutes.

Number	1.15 P.M.	1.30 P.M.	pH Optimum range
Bottle 1	Breaking up	Partly dissolved	—
Bottle 2	Partly dissolved	Fully dissolved	9.10
Bottle 3	Slightly attacked	Fully dissolved	—
Bottle 4	Slightly attacked	Fully dissolved	—
Bottle 5	Slightly attacked	Partly dissolved	8.02
Bottle 6	Unaltered	Broken up	—

Instead of more or less neutralising the 2 per cent. HCN solution by Na_2CO_3 , varying quantities of 5 per cent. KCN solution were next employed. It may be noted that KCN formula weight is 65, which is just about two and a half times the formula weight 27 of HCN. So that 5 per cent. KCN solution is equivalent to 2 per cent. HCN in CN content.

On April 2, 1931, juice was pressed from fresh unripe

papaw fruit. The juice was centrifuged and a clear liquid obtained, which came to about 66 per cent. of the fruit pulp taken. The juice was taken in different bottles, as detailed below, and placed in the incubator for preliminary raising of the temperature at 12.30 P.M.

Bottle 1 contained 40 c.c. of juice with 1 c.c. of 5 per cent. KCN solution.

Bottle 2 contained 40 c.c. of juice with 2 c.c. of 5 per cent. KCN solution.

Bottle 3 contained 40 c.c. of juice with 4 c.c. of 5 per cent. KCN solution.

Bottle 4 contained 40 c.c. of juice with 4 c.c. of 5 per cent. KCN solution.

Bottle 5 contained 40 c.c. of juice *without any* KCN solution.

After the preliminary incubation for half an hour in the incubator, into each one of the bottles was introduced a shred of fibrin (of about equal weight) at 1 P.M. The bottles were then replaced in the incubator and observations taken every 30 minutes.

Number	1.30 P.M.	2 P.M.	2.30 P.M.	Optimum range
Bottle 1	Broken up	Partly dissolved	Fully dissolved	—
Bottle 2	Dissolved	Fully dissolved	Fully dissolved	pH 8.20
Bottle 3	Broken up	Partly dissolved	Fully dissolved	—
Bottle 4	Unattacked	Unattacked	Broken up	pH 9.70
Bottle 5	Unattacked	Unattacked	Unattacked	—

A comparison of the results obtained on April 1 and 2, 1931, as summarised in the two foregoing tables, shows that dissolution of fibrin is effected through a wide range of values. The quickest reaction, however, is dependent on the concentration of CN in the solution. The best concentration is about 1 grm. per litre, the pH value being between 8.2 and 9.2.

SUMMARY

(1) Finkler's papain seems to be very nearly pure peptase; it resembles that obtained by kaolin treatment, but is more like the papain obtained by precipitation in alcohol. Some samples contain a little ereptase: this is more often the case with the ordinary commercial though whiter variety of market papain. Extraction in distilled water gives rather a weak solution of peptase; a stronger and more effective solution as regards fibrin dissolving power is obtained when NaCl solution is used for extraction. The fact that no relation could be detected between fibrin-digesting and the peptone-splitting activities offers additional evidence that papain contains two distinct proteolytic enzymes, peptic and ereptic, of which the former is the more predominant.

(2) Fibrin-dissolution is very much quicker in the presence of the CN ion, in an adequately alkaline medium. The most rapid fibrin-dissolution was observed when the pH value was between 8.2 and below 9.2, with a CN concentration of 1 grm. per litre or one-gram-formula-weight per 26 litres. This amounts to about 3.9×10^{-2} M. (or if ONE c.c. were taken as the unit of volume, 3.9×10^{-5} M.).

We take this opportunity of expressing our grateful thanks to Sir J. C. Bose and Professor S. H. Vines for their kind interest and encouragement throughout the investigations.

ADDITIONAL BIBLIOGRAPHY

- HALDANE, J. B. S.: *Enzymes*, 1930, pp. 143, 144.
 NORD, F. F.: 'The So-called Activation,' *Mechanism of Enzyme Action and Associated Cell Phenomena*, 1929, chap. iv.

XV.—CHEMICAL EXAMINATION OF OILS FROM LEGUMINOUS PULSES

BY

H. N. BANERJEE, M.Sc.

IN the course of a previous investigation on the composition of some of the common leguminous pulses in Bengal, and of the nature of the proteolytic enzymes present in them, certain interesting peculiarities were observed in some of them. From amongst these, a few analytical results have already been published.¹

The seeds of *Cicer arietinum* (*Chhola*, *Boot*, *Chana*) contain a higher percentage of oil than the other common pulses, proteid nitrogen being comparatively less. Certain peculiarities were noticed as regards the presence of tryptophane and enzymes in the watery or NaCl solution extract of the ungerminated seeds of *Cicer arietinum* and of *Phaseolus radiatus*—particularly the variety *Shona Moog*.²

The subject of the present Paper is a more thorough examination of the oil from the seed of *Cicer arietinum*. The proportion of oil is about 4 to 5 per cent. In this our results agree with those of Dr. Leather, late of the Indian Agricultural Department. A slight variation was to be expected, due to climatic condition and the locality where the plants were grown.³

I. *CICER ARIETINUM*

Method of oil extraction.—The method followed for the extraction of the oil was the ordinary Ether Extraction

¹ N. C. Nag, H. N. Banerjee, and K. Bose, *Proc. XVIIth Indian Sc. Congress*.

² N. C. Nag and H. N. Banerjee, *Trans. Bose Inst.*, 1930-31, vol. vi.

³ S. Ivanow, *Die Klimaten des Erdballs und die Chemische Tätigkeit der Pflanzen* (quoted by Harvey, *Plant Physiology*).

Method in Soxhlet from the dried and powdered seed, the details of which are well known. I took special precautions to drive off the ether and dry the oil without unduly exposing it to the free air, a vacuum desiccator having been used for the purpose.

After extraction of the oil, the ordinary constants, such as specific gravity, saponification value, iodine value (Hanus), refractive index (by Abbe Refractometer), percentage of fatty acid, unsaponifiable matter, Reichert-Meissl number, acid value, coefficient of expansion, as well as a few other factors, were determined by the usual methods. In the two following tables I give in the first horizontal rows the values obtained at the Bose Research Institute. In the second rows are given the results obtained by Grimme.¹

TABLE XXXII.—PHYSICAL CONSTANTS (CHHOLA OIL).

Oil yield	Sp. gr.	Refractive index	Coefficient of expansion
4.2 per cent.	0.9306 at 30° C.	1.4780 at 29° C.	0.00052 (30° C. to 100° C.)*
5.1 per cent.	0.9184 at 15° C.	1.4717 at 30° C.	

* The coefficient of expansion was determined by Professor N. C. Nag.

TABLE XXXIII.—CHEMICAL CONSTANTS (CHHOLA OIL).

Saponification value	Iodine value	Fatty acid	Unsaponifiable matter	Acid value	Reichert-Meissl number
184.2	129-130	90 per cent.	1.5 per cent.	4.2	8.6
182.6	118.5	92.62 per cent.	1.08 per cent.	—	—

¹ Grimme, *Pharm. Zentralb.* (1911), 1141.

My results will be seen to be very near those of Grimme, taking into account the fact that of the two sets of values one was obtained in Asia and the other in Europe.

MIXED FATTY ACIDS

A weighed quantity of the oil was fully saponified with alcoholic potassium hydroxide. The residual alcohol was evaporated off and the fatty acids liberated by acidification with dilute sulphuric acid. The liberated fatty acids were then extracted with ether. The ether was then driven off, and the result showed 90 per cent. fatty acid. The following constants for the mixed fatty acids were determined, the first row in the table giving results obtained by me, while the second row relates to the values obtained by Grimme.

TABLE XXXIV.—CHEMICAL AND PHYSICAL CONSTANTS OF FATTY ACID MIXTURE (CHHOLA OIL).

Neutralisation value	Mean molecular weight	Iodine value (Hanus)	Refractive index
199·2	281	140	1·4685 at 32° C.
189·4	296	120·3	1·4587 at 40° C.

SEPARATION OF THE UNSATURATED FROM THE SATURATED ACID (CHHOLA OIL)

Of the different methods for separating the liquid unsaturated from the solid saturated fatty acids, I followed Gusserow Varrentrapp's Lead-salt-ether method,¹ as also that of Twitchell's Lead-salt-alcohol method.² Details of the methods will be found in the literature quoted below. My experience is that Twitchell's method, though a little

¹ Lewkowitsch, *Oils and Fats*, vol. i, p. 556.

² Twitchell, *Jour. Ind. Eng. Chem.*, 1921, vol. xiii, p. 806.

more laborious, gives a better separation. For example, by Varrentrapp's method the yield of solid acid was 12 per cent., in which I found the iodine value to be 40. According to Twitchell, on the other hand, the percentage yield was 10, with a much lower iodine value of 30.

The chemical and physical constants of the separated acids are tabulated below.

TABLE XXXV.—CHEMICAL AND PHYSICAL CONSTANTS OF SEPARATED ACIDS (CHHOLA OIL).

Method	Solid saturated acids			Liquid unsaturated acids		
	Yield	Iodine value	Melting point	Yield	Iodine value	Refractive index
Varrentrapp	Per cent. 12	40	50° C.	Per cent. 88	142.3	1.4700 at 29° C.
Twitchell	10	30	55° C.	90	145	1.4680 at 30° C.

IDENTIFICATION OF LIQUID ACIDS AS BROMO-DERIVATIVES

For the purpose of separating the liquid from the solid fatty acids, the process worked out by Tortelli and Ruggeri¹ was resorted to. After separation of the liquid from the solid, the different constituents of the liquid acid-mixture were separated as their bromo-derivatives. For this 3.15 grms. of the separated liquid unsaturated acid were brominated in ether solution at -10° C., according to the *modus operandi* recommended by Eibner and Muggenthaler.²

LINOLENIC ACID

The ether-insoluble solid formed after bromination was washed repeatedly with *chilled ether* until the washings were colourless. The solid thus obtained was dried at 100° C. for

¹ Tortelli and Ruggeri (Lewkowitsch, *Oils and Fats*, vol. i, p. 560).

² Eibner and Muggenthaler, *Farben Zeitung*, 1912, No. 3 ff.

two hours to constant weight. In the following line are given the yield and its melting point :

Yield, 0.2 ; melting point, 177° C.

The bromine content in the bromo-derivative was estimated by Schiff's method and found to be 63.36 per cent. The formula $C_{18}H_{30}O_2Br_6$ (Linolenic Hexabromide) requires the bromine content to be 63.32 per cent.

LINOLIC ACID

The mother liquor from the ether insoluble hexabromide was then repeatedly washed with sodium-thiosulphate in a separator to remove excess of bromine. The ether solution was finally filtered through a dry filter to remove adhering moisture. The ether was next distilled off and the residue dissolved in boiling *petroleum ether*. On cooling the petroleum ether beautiful white crystals separated out, which proved on analysis to be linolic tetrabromide.

Yield, 4.00 grms. ; melting point, 113° C.

The bromine content was estimated and found to be 53.71 per cent., the formula $C_{18}H_{32}O_2Br_4$ (Linolic Tetrabromide) requiring 53.33 per cent.

OLEIC ACID

The mother liquor, after filtering off the crystals of linolic tetrabromide from petroleum ether, was evaporated down and a deep brown oily substance obtained.

Yield, 1.9 grms.

The bromine content was estimated and found to be 36.6 per cent., the formula $C_{18}H_{34}O_2Br_2$ (Oleic Dibromide) requiring 36.18 per cent.

Calculating the percentages of the individual liquid unsaturated acids—*viz.* oleic, linolic, and linolenic—from the yields of the respective bromo-derivatives noted above, the

following quantitative relations expressed in tabular form are obtained :

TABLE XXXVI.—PERCENTAGES OF ACIDS.

Name	Yield	Calculated acid	Percentage of individual acid
Oleic Dibromide	1.90	1.21	38.41 Oleic Acid
Linolic Tetrabromide	4.00	1.87	59.36 Linolic Acid
Linolenic Hexabromide	0.20	0.07	2.23 Linolenic Acid

UNSAAPONIFIABLE MATTER

After separation and identification of the principal constituents of the liquid unsaturated acids present in the *Cicer arietinum* oil I proceeded to examine the unsaponifiable matter. This was obtained from the oil by the following procedure :

A quantity of oil was saponified with alcoholic potassium hydroxide. The mass was then heated on the water bath to drive off the bulk of alcohol and the soap dissolved in water. The product was then repeatedly extracted with ether in a stoppered separating funnel until the ether extract was colourless. The ethereal solution was washed thoroughly with distilled water to get rid of any adhering soap. The ether was then driven off and the coloured residue dissolved in the minimum quantity of boiling alcohol (98 per cent.) ; on cooling, crystals of solid needles grouped in tufts separated out. Under the microscope they were observed as long solid needles arranged in star-like groups. These colourless crystals were identified as Phytosterol, which melted at 135° C.–137° C. The melting point of Phytosterol from different sources gives different values from 135° C. to 144° C.¹

¹ Leathes and Raper, *The Fats: Monographs on Biochemistry*, p. 50.

CAROTINOID PIGMENTS

The alcoholic mother liquor, after the Phytosterol had been separated, was strongly yellowish-orange in colour with characteristic absorption bands. After evaporation of the alcohol there was left a small quantity of orange-coloured residue rather easily soluble in carbon bisulphide. At the suggestion of Professor N. C. Nag, I found the material to consist of carotinoid pigments.¹

As has already been noted, even ungerminated seeds of *Cicer arietinum* contain water extractable proteolytic enzymes, as also tryptophane, indicating easy digestibility. The presence of carotinoid pigments gives additional interest from the vitamin point of view.²

2. OIL FROM *PHASEOLUS RADIATUS* (SHONA MOOG)

The following deals with the chemical examination of oil from seeds of the golden-yellow variety of *Phaseolus radiatus*—*Shona Moog*. The general line of procedure was the same as that followed in the previous paper on the oil from *Cicer arietinum*. There are several varieties of pulses going by the same name Moog; the variety *Shona Moog* is dealt with here, future communications being reserved for the other varieties.

The dry seeds of *Phaseolus radiatus*, variety *Shona Moog*, yielded by ether extraction an oil of deep yellowish-green colour, with a characteristic sweet odour. The following constants were determined :

TABLE XXXVII.—CHEMICAL AND PHYSICAL CONSTANTS
(*SHONA MOOG OIL*).

Oil yield	Iodine value (Hanus)	Saponi- fication value	Unsaponifiable matter	Refractive index
1 per cent.	102.2	185	1.8 per cent.	1.4820 at 30° C.

¹ H. Molisch, *Microchemie der Pflanze*, 1923, pp. 252-256.

² Sherman and Smith, *Vitamins* (1931 ed.), p. 243 ('Probable Relationship of Vitamins to Certain Plant Pigments,' Steenbock and others).

MIXED FATTY ACIDS

A weighed quantity of oil was saponified by means of alcoholic potash and the resulting soap solution repeatedly washed with small quantities of ether to remove the unsaponifiable matter. From the soap solution the fatty acids were liberated, as in the previous case of *Cicer arietinum*; the following constants were determined:

TABLE XXXVIII.—CHEMICAL AND PHYSICAL CONSTANTS OF FATTY ACIDS (SHONA MOOG).

Free acid yield	Iodine value (Hanus)	Neutralisation value	Mean molecular weight	Refractive index
90 per cent.	106.5	200	280	1.4720 at 30° C.

SEPARATION OF THE UNSATURATED FROM THE SATURATED FATTY ACID

A weighed amount of the oil was saponified by alcoholic potash, and the bulk of the alcohol distilled off and the soap dissolved in water. The unsaponifiable matter was then completely removed by repeated ether extraction as detailed in the previous Paper, and then the fatty acid mixture was liberated by the usual method, which was then treated according to Twitchell process.¹

The percentages of the saturated solid and the unsaturated liquid acids, with their respective chemical and physical constants, are given below.

¹ Twitchell, *Jour. Ind. Eng. Chem.*, 1921, vol. xiii, p. 806.

TABLE XXXIX.—CHEMICAL AND PHYSICAL CONSTANTS OF SEPARATED ACIDS (*SHONA MOOG OIL*).

Solid saturated acids			Liquid unsaturated acids		
Yield	Melting point	Iodine value (Hanus)	Yield	Iodine value (Hanus)	Refractive index
25 per cent.	50° C.— 51° C.	47·7	75 per cent.	126·5	1·4750 at 28° C.

IDENTIFICATION OF LIQUID ACIDS AS BROMO-DERIVATIVES

The liquid fatty acid mixture, as obtained by Twitchell process, was subjected to bromination at -10°C . in dry ether solution according to Eibner and Muggenthaler,¹ as detailed under the previous Paper on *Cicer arietinum* oil. Two grams of the liquid unsaturated acid mixture were taken for this purpose.

LINOLENIC ACID

The ether insoluble solid formed after bromination was repeatedly washed with *chilled ether* until free from adhering bromine, and then dried at 100°C . for several hours until constant weight.

Yield, 0·1 grm. ; melting point, 180°C .

Bromine content was estimated and found to be 63·0 per cent. The formula $\text{C}_{18}\text{H}_{30}\text{O}_2\text{Br}_6$ (Linolenic Hexabromide) requires 63·32 per cent.

LINOLIC ACID

The mother liquor from the ether-insoluble hexabromide was repeatedly washed with sodium-thiosulphate solution and then with distilled water. The ether from the

¹ Eibner and Muggenthaler, *Farben Zeit.*, 1912, No. 3 ff.

etheral solution was next driven off and the residue was then dissolved in boiling petroleum ether. On cooling, linolic tetrabromide separated out. After filtration the solid residue was dried and weighed.

Yield, 1.47 grm. ; melting point, 111° C.— 113° C.

Bromine content was estimated and found to be 53.63 per cent. The formula $C_{18}H_{32}O_2Br_4$ (Linolic Tetrabromide) requires 53.33 per cent.

OLEIC ACID

The petroleum ether solution, after removal of the linolic tetrabromide, was evaporated to dryness and a deep brown oily mass was obtained.

Yield, 2 grms.

Bromine content was estimated and found to be 36.44 per cent. The formula $C_{18}H_{34}O_2Br_2$ (Oleic Dibromide) requires 36.18 per cent.

Calculating the percentages of the individual liquid unsaturated acids—*viz.* oleic, linolic, and linolenic acids—from the yields, noted above, of their respective bromo-derivatives, the following relations expressed in tabular form are obtained :

TABLE XL.—PERCENTAGES OF ACIDS.

Name	Yield	Calculated acid	Percentage of individual acid
Oleic Dibromide	2.00	1.274 grm.	63.7 Oleic Acid
Linolic Tetra-bromide	1.47	0.6893 grm.	34.5 Linolic Acid
Linolenic Hexa-bromide	0.10	0.0367 grm.	1.8 Linolenic Acid

UNSAPONIFIABLE MATTER (*SHONA MOOG OIL*)

The unsaponifiable matter, which was 1·8 per cent. of the oil, was dissolved in boiling alcohol (98 per cent.). On cooling, a Phytosterol crystallised out, which was further purified by a second crystallisation. The Phytosterol then melted at 133° C.–135° C.

Carotinoid pigments were detected in the mother liquor after sterol separation.¹

Phaseolous radiatus seed—particularly the variety *Shona Moog*—resembles *Cicer arietinum* in many respects as regards digestibility and vitamin content.

I take this opportunity of expressing my gratefulness to Sir J. C. Bose for constant encouragement and to Professor N. C. Nag for valuable suggestion and guidance.

ADDITIONAL BIBLIOGRAPHY

1. BERTIER, *Semis. Enciclop. Chim.*, 4, 445.
2. RITTER, *Bried. Centr.*, 1884, 529.
3. PASSERINI, N., *Staz. Sper. Agrar.*, 21, 20–30.
4. CASTRO, N., *Gazetta*, 1909, 39, i, 608–625.
5. ZLATAROFF, *Zeitsch. Nahr. Genuss.*, 1916, 31, 180–183.
6. BURR, M. M. and G. O., *Jour. Biol. Chem.*, 1930, pp. 587–621.
7. WILLSTÄTTER and STOHL, *Investigations on Chlorophyll* (English translation by Schertz and Merz).

¹ H. Molisch, *Microchemie der Pflanze*, 1923, pp. 252–256.

XVI.—A COMPARATIVE STUDY OF BURMESE CRANIA

BY

PROVASH CHANDRA BASU, M.B., M.Sc., P.R.S.

THE following account is based on the collection of the Burmese Crania, obtained from an old burial-ground in Prome, that are at present in the collection of the Anthropological Section of the Indian Museum, Calcutta, being procured on two separate occasions—the skulls Nos. 421-437 in December 1868, and the skulls Nos. 450-468 and 495 in March 1869. Unfortunately the records kept in the Indian Museum give no details as regards their antiquity and the manner of their burial. A careful examination of the skulls, however, leaves no room for doubt that no high antiquity can be attributed to any of these skulls, all of which must be of a recent period. The late Mr. B. A. Gupte¹ published in 1909 a few measurements only on these skulls; but as this series is regarded as an extremely valuable one, reliably known Burmese skulls being very rare, I undertook, on the advice of Dr. B. S. Guha, Officer-in-Charge of the Anthropological Section of the Indian Museum, a detailed study of this series, so that accurate data may be available for purpose of comparison with the other Mongoloid races of the country. In taking the measurements I have followed strictly the procedure laid down in the International Agreement. All anatomical points, which are used in taking craniometric measurements, were first marked with a pencil so as to ensure uniformity in the measurements as far as possible.

The skulls are altogether 36 in number. Of these, 30

¹ *Craniological Data from the Indian Museum, Calcutta*, pp. 66-70 (Calcutta, 1909).

appear to belong to the male and 6 to the female sex. The mandibles are absent, except in 10 skulls. The bones are in good condition and are of a pale whitish colour.

THE AGE

Judging from the eruption of the secondary teeth and the nature of the suture closure, the skulls appear to belong to individuals of varying ages. Only one skull (No. 452) seems to be below twenty years of age, for in this the eruption of the teeth has not been complete (there are only 14 teeth in the upper jaw) and the basioccipital has not fused with the basisphenoid.

Recent researches of T. Wingate Todd and D. W. Lyon, Jr.,¹ have shown that the closure of the ectocranial sutures of the cranial vault offers, to a considerable extent, materials for determining the age of the individual. The results of their investigations indicate that there is one modal type of human suture closure which is unaffected by race and stock. An attempt has been made here to utilise their results, together with the evidence obtained from dentition and closure of the basicranial sutures, to estimate the probable age of some of the skulls of the particular series under examination.

Thus, in skull No. 422, the third molar has erupted, but the basioccipital has not fused with the basisphenoid, while the closure has started at Pars pterica of the coronal suture which, according to Wingate Todd and Lyon, commences at 22 years; the closure has also started at Pars obelica of the sagittal suture, which usually starts at 20 years, but it has not commenced at Pars lambdoidica and at Pars verticis. All these confirm the conclusion that the individual was about 20 years of age. In the skulls Nos. 427, 457, and 463, the dental sockets in the upper jaw show that there were 16 teeth present, but neither the sutures of the cranial vault nor the basioccipito-sphenoid suture

¹ T. Wingate Todd and D. W. Lyon, Jr., 'Cranial Suture Closure, its Progress and Age Relationship,' pt. ii, *Amer. Journ. Phys. Anthropol.*, 1925, vol. viii, pp. 23-71.

show any sign of union. Evidently, therefore, the skulls numbered 422, 427, 457, and 463, belong to individuals between 20 and 25 years of age.

The rest of the crania, which are 31 in number, have their third molars and the basioccipito-sphenoid suture closed completely, showing that they were over 25 years of age.

CRANIAL CAPACITY

The cranial capacity, calculated according to the Formulae Nos. 10 and 11 of Alice Lee and Karl Pearson,¹ shows a range of variability from 1298 to 1661 c.c. in the males and from 1204 to 1345 c.c. in the females; the mean cranial capacity in the males is therefore 1450 ± 9.87 c.c. and in the females 1279 ± 14.60 c.c. respectively. In the skulls measured by Tildesley² the average cranial capacity given is as 1406.9 (27), 1415.0 (4), and 1442.2 (5) c.c. for the Groups A (Burmese Proper), B (Hybrid), and C (possibly Karens) respectively in the males, and 1267.9 (27), 1232.4 (11), and 1231.4 (7) c.c. for the Groups A, B, and C respectively in the females. In the above the figures within brackets indicate the number of specimens examined. The average cranial capacity of the 28 male Burmese crania proper obtained from Prome, Tharawaddy, Hanthawaddy, Ava, etc., and measured by Sir William Turner,³ was 1388 c.c.; of these, one was 1160 c.c., two 1600 and 1670 c.c., and one 1820 c.c. Thus, compared with the figures of Tildesley's Burmese Proper Group A as well as with the Burmese Proper of Sir William Turner, we find that the average capacity in our series is greater by 43.1 c.c. from Tildesley's Group A and by 62 c.c. from those measured by Turner. But both Tildesley and Turner obtained the cranial capacity of their series by

¹ Alice Lee and Karl Pearson, 'A First Study of the Correlation of the Human Skulls,' *Phil. Trans. Roy. Soc.*, Series A, vol. cxcvi, p. 247 (London, 1901).

² M. L. Tildesley, 'A First Study of the Burmese Skull,' *Biometrika*, vol. xiii, pp. 176-262 (Cambridge, 1920-21).

³ Sir William Turner, 'Contributions to the Craniology of the People of the Empire of India,' pt. i; 'The Hill Tribes of the North-East Frontier and the People of Burma,' *Trans. Roy. Soc. Edin.*, vol. xxxix, pp. 700-747 (Edinburgh, 1900).

direct measurement, and in the present case it is obtained by calculation according to the Lee-Pearson Formulae. The difference between the three results may therefore be regarded as practically negligible.

GENERAL CHARACTERISTICS OF THE SKULLS

The position of the External Auditory Meatus in the skull has not hitherto received the attention it deserves. Following the suggestion of Sir Arthur Keith¹ that it would be well worth while to regard this as a racial trait, Sewell and Guha² have devised a method of calculating the Meatal Position Index (M.P.I.) according to the following formula :

$$\text{M.P.I.} = \frac{\text{Nasion to foot of meatal perpendicular} \times 100}{\text{Nasioninion length}}$$

Calculated according to this method the average Meatal Position Index in the present series is 55.5 ± 0.31 in the males and 55.8 ± 0.36 in the females. The average Meatal Position Index obtained by Sewell and Guha³ in three adult skulls from Mohenjodaro is 48.1 , in 5 Veddahs 51.43 , in 20 Tasmanians 51.49 , in 20 Australians 53.01 , and in 3 Aditannalur skulls 54.06 , showing thereby that in the Burmese series the posterior parts of the skull are not projected so much backwards as in the skulls mentioned above.

In the present series of Burmese skulls, moreover, the supra-orbital ridges are only moderately developed in the males, and the forehead is sometimes retreating. The supra-orbital ridges are, however, never so strongly developed as to form a supra-orbital torus as in the Australian, Tasmanian, and Melanesian skulls, or even as much as in some of the Naga skulls recently studied.⁴

¹ Sir Arthur Keith, 'Reports on Two Crania of considerable but uncertain Antiquity,' *Jour. Anthropol. Soc. Bombay*, vol. xi, p. 671 (Bombay).

² R. B. S. Sewell and B. S. Guha, *Report on the Human Remains excavated at Mohenjodaro*—Mohenjodaro and the Indus Valley Civilisation, edited by Sir John Marshall, vol. ii, chap. xxx, p. 607 (London, 1931).

³ R. B. S. Sewell and B. S. Guha, *loc. cit.*, p. 607.

⁴ B. S. Guha and P. C. Basu, 'A Report on the Human Relics recovered by the Naga Hills (Burma) Expedition for the Abolition of Human Sacrifice during 1926-27,' *Anthropological Bulletin No. 1, Zoological Survey of India*, p. 15 (Calcutta, July 1931).

<i>Coronal Suture:</i> Pars bregmatica	6 I ₁ , 9 I ₂ , and 1 I ₃	4 II ₂ , 4 II ₃	5 III ₂ , 4 III ₃	1 IV ₄ , 1 IV ₅
	2 I ₃ , 8 I ₄	1 II ₂ , 3 II ₃ , 1 II ₄ , 1 II ₇ , 1 II ₈	4 III ₂ , 1 III ₃ , 2 III ₆ , 1 III ₈	1 IV ₂ , 2 IV ₄ , 1 IV ₅ , 2 IV ₆ , 1 IV ₇ , 1 IV ₈ , 1 IV ₉ , and 1 agreeing with No. 2 of fig. 322 *
Pars temporalis	18 I ₁ , 12 I ₂ , and 1 I ₃	1 II ₃	1 III ₁	1 IV ₇
<i>Sagittal Suture:</i> Pars bregmatica	4 I ₂ , 6 I ₃ , and 1 I ₄	1 II ₂ , 10 II ₃ , and 1 II ₅	7 III ₂	1 IV ₅ , 1 IV ₈
	1 I ₃ , 3 I ₄ , and 1 I ₉	5 II ₂ , 3 II ₃ , 12 II ₆ , 3 II ₇ , and 2 II ₈	1 III ₅	
Pars verticis	13 I ₂ , 13 I ₃ , and 1 I ₄	1 II ₂ , 1 II ₃ , 1 II ₅ , 1 II ₆	1 III ₆	
Pars obelica	6 I ₃ , 1 I ₄ , and 1 I ₅	3 II ₂ , 4 II ₃ , 6 II ₆ , 1 II ₈	6 III ₂ , 2 III ₇ , and 2 III ₈	1 IV ₆ , 2 IV ₇ , and 2 IV ₈
<i>Lambdaoid Suture:</i> Pars lambdaeidea	2 I ₂ , 4 I ₃ , 2 I ₅ , 1 I ₈ , and 1 I ₉	6 II ₂ , 4 II ₄ , 1 II ₅ , 3 II ₆	1 III ₁ , 1 III ₄ , 3 III ₆ , 2 III ₈ , and 2 III ₉	1 IV ₄
	1 I ₄ , 1 I ₆ , and 1 I ₉	1 II ₂ , 2 II ₄ , 3 II ₅ , 1 II ₆ , 1 II ₇ , 1 II ₈ , 1 II ₉	2 III ₄ , 3 III ₅ , 6 III ₆ , 2 III ₇ , 1 III ₁₀	2 IV ₆ , 4 IV ₈ , 1 IV ₁₀
Pars asterica	4 I ₁ , 20 I ₂ , 3 I ₃ , 1 I ₅	2 II ₃	1 III ₁ , 2 III ₂	1 IV ₈ , 1 IV ₉

Explanation of numbers in the table:

(After Martin's 'Lehrbuch der Anthropologie,' Bd. II, Jena, 1928.)

The headings of columns refer to the main or principal group of suture characters of Oppenheim. An ordinary numeral before the main group means number of specimens examined. The numeral a little below the group number means subdivision of that group.

* This cranium differs very slightly from Group IV, but agrees more closely with No. 2 of fig. 322 given in p. 734 of above book by Martin.

A few of the skulls present a considerable degree of asymmetry in the parietoccipital regions. This asymmetry has also been noted both by Turner¹ and by Tildesley.²



FIG. 150. Brachycranial chamaerrhine.
♀ $\times \frac{1}{3}$ (skull No. 426).

Epipteric bones are present in five skulls. Wormian bones are often present along the course of the lambdoid suture—usually they are small, and only in one skull is it large in

¹ Sir William Turner, *loc. cit.*, p. 729.

² M. L. Tildesley, *loc. cit.*, pp. 251 ff.

size. The character and serrations of the sutures are given in Table XLI, arranged according to the classification of Oppenheim.¹

The skulls are, in the main, brachycranial, but four show dolicho characters (*vide* figs. 150–155). In figs. 150 and 151 are given the brachycranial chamaerrhine type of skulls which form the dominant type; the first is that of a female and the second that of a male. The rest of the series of figures are illustrative of the intermediate and the long-headed types. Of these, fig. 152 represents the brachycranial leptorrhine type, fig. 153 is that of the mesocranial chamaerrhine group, fig. 154 gives the dolichocranial chamaerrhine skull, and fig. 155 exhibits the dolichocranial leptorrhine type. In all the above figures the upper left illustration is the front view, the lower illustration of the series is the side view, while the upper-right illustration is the view from the top of the skull. In the Tables XLII–XLV is given a full list of measurements with their indices and statistical constants for both the sexes. The average maximum cranial length is 173.3 mm. for ♂ and 165.0 for ♀, and the maximum cranial breadth is 141.1 mm. for ♂ and 134.5 for ♀. The mean length-breadth index is 81.4 ± 0.62 for ♂ and 81.1 ± 0.89 for ♀, and the Standard Deviation is 5.04 ± 0.44 for ♂.

In the skulls measured by Tildesley² the average maximum cranial length in Group A (Burmese Proper) is 173.5 for ♂ and 166.5 for ♀, and maximum cranial breadth is 143.7 for ♂ and 138.0 for ♀. The average cranial index in Group A (Burmese Proper) is 82.9, in Group B (Hybrid) 80.4, in Group C (Karens?) 79.8, in the males; whereas the index is 83.1, 82.9, and 76.9 in females for the three groups respectively.

In the Burmese crania proper measured by Sir William Turner,³ we find the average maximum cranial length to be 172.8 and the average maximum cranial breadth 141.7 mm. The average length-breadth index is 82.1. There are

¹ R. Martin, *Lehrbuch der Anthropologie*, Bd. II, S. 732–733 (Jena, 1928).

² M. L. Tildesley, *loc. cit.*, p. 219.

³ W. Turner, *loc. cit.*, p. 734.

2 crania with indices below 75, and of the 10 mesocranial skulls 8 are above 77.5. Of the rest, 8 have a cranial index of 85 or upwards, of which 2 are as high as 90.



FIG. 151. Brachycranial chamaerhine.
 $\sigma \times \frac{1}{4}$ (skull No. 432).

Compared, therefore, with those just mentioned we find that the maximum cranial length and the maximum cranial breadth in all the three series agree very closely, the differ-

ences being within 3 mm. The mean cranial index in the males of the present series is 1.5 and 0.7 unit lower than that of Tildesley and Turner respectively, and in the females it is 2.0 units lower than that of Tildesley. The differences are not marked enough to have any significance and we may regard all the three to be closely similar.

The skulls are usually high-vaulted, the average auricular height being 122.0 mm. for ♂ and 118 mm. for ♀. The average basibregmatic height is 134.6 for ♂ and 128 for ♀, and the average length-height index (basibregmatic) is 77.6 for ♂ and 77.9 for ♀.

The average auricular height given by Tildesley of Burmese Group A is 117.7 for ♂ and 111.7 for ♀. It is possible that the considerable difference noticed between the two series may have been due to differences in the technique followed.¹ Tildesley has taken the basibregmatic height from 'basion to a point vertically above it a little behind the bregma,' and in Burmese Group A the average given by the author is 136.8 for ♂ and 131.4 for ♀. The average length-height index is 78.5 for both ♂ and ♀.

Turner has not given the auricular height. The average basibregmatic height given by Turner is 135.1 and the length-height index is 78.2.

Compared, therefore, with the results arrived at by Turner and Tildesley, we find the differences in basibregmatic height to be only 0.5 mm., but Tildesley's Group A shows somewhat greater height for both ♂ and ♀. The length-height index in both Turner and Tildesley also is slightly higher. As compared, therefore, with Turner's and Tildesley's figures the skulls of this collection, though agreeing closely in respect of length and breadth, are somewhat lower vaulted.

The nose is predominantly broad. The average nasal length is 51.2 mm. for ♂ and 48.5 mm. for ♀, and the average nasal breadth is 26.7 for ♂ and 26.5 for ♀. The mean nasal index is 52.5 ± 0.66 for ♂ and 54.7 ± 1.40 for ♀.

In Tildesley's² Group A the average nasal length is

¹ M. L. Tildesley, *loc. cit.*, p. 181.

² M. L. Tildesley, *loc. cit.*, pp. 219-220.

53.1 mm. for ♂ and 50.6 mm. for ♀, and the average nasal breadth is 28.1 mm. for ♂ and 26.8 for ♀. The average nasal index obtained is 52.7 in Group A, 51.0 in Group B,



FIG. 152. Brachycranial leptorrhine.
♂ $\times \frac{1}{3}$ (skull No. 454).

and 46.3 in Group C in males, and 53.0, 50.7, and 50.4 for the Groups A, B, and C respectively in the females.

In the Burmese crania measured by Turner¹ the average

¹ W. Turner, *loc. cit.*, p. 735.

nasal length is 52 mm. and the average nasal breadth 25.3, the average nasal index being therefore 48.6. The range of variability is between 40.0 to 59.1.



FIG. 153. Mesocranial chamaerrhine.
♂ $\times \frac{1}{3}$ (skull No. 462).

Compared with the figures given by Tildesley, the average nasal length of the present series is shorter by 2.3 mm. in the ♂ and 2.1 mm. in the ♀, and the average nasal breadth by 1.4 mm. in the ♂ and 0.3 mm. in the ♀. The difference

in the average nasal index in the males is very slight, being only 0.2, but is slightly higher in the females, *i.e.* 1.7 units. Compared with the figures published by Turner, however, there is an appreciable difference in the nasal index, the average nasal index being 3.9 units higher in our series. This higher index is due not so much to the difference in the nasal length, which is only 0.8 mm., but to the greater nasal breadth, which is 2.8 units higher in our series.

Coming to the orbits, we find that the orbits at the two sides show some degree of variability. They are usually medium in size, though large and small orbits are also present. The mean orbital index in the male is 80.6 ± 0.64 on the right side and 82.5 ± 0.73 on the left side, whereas in the females it is 86.3 ± 1.38 on the right side and 85.6 ± 1.76 on the left. In the skulls measured by Tildesley¹ we find the average orbital index to be as follows :

Groups	A		B		C	
	♂	♀	♂	♀	♂	♀
Right	79.1 (41)	82.4 (34)	75.9 (8)	80.4 (16)	80.3 (7)	79.9 (14)
Left	80.0 (41)	82.8 (34)	79.4 (8)	83.0 (14)	81.5 (7)	78.8 (16)

Compared with Tildesley's Group A, therefore, we find the mean orbital index of the present series to be higher for ♂ by 1.5 units on the right side and by 2.5 units on the left side.

In the Burmese crania proper measured by Sir William Turner² the average orbital index given is 85.0, and the

¹ M. L. Tildesley, *loc. cit.*, p. 219.

² W. Turner, *loc. cit.*, p. 735.

range of variation is from 73·2 to 97·3. He has not, however, distinguished between the orbits of the two sides.



FIG. 154. Dolichocranial chamaerrhine.
♂ $\times \frac{1}{3}$ (skull No. 495).

Compared with the figures published by him the average orbital index in the present series is 4·4 units lower on the right side and 2·5 units on the left.

The zygomatics are prominent and the face is characteristically flat. The maximum bizygomatic breadth is



FIG. 155. Dolichocranial leptorrhine.
 $\sigma \times \frac{1}{3}$ (skull No. 460).

131.2 mm. for σ and 124.6 for φ . In Turner's series it is 134 mm., whereas in Tildesley's Group A it is 134.0 mm. for σ and 126.7 for φ , showing that the average bizygomatic

breadth of our series is 2.8 mm. shorter in the males than those of Turner and Tildesley.

The teeth are usually slightly eroded in their crowns, and in one skull there are signs of caries.

The palates are usually broad. The average palatal length is 43 mm. for ♂ and 42.6 mm. for ♀, and the average palatal breadth is 40 mm. for ♂ and 36 for ♀. In Tildesley's Group A the average palatal length is 49.9 for ♂ and 46.7 for ♀, and the average palatal breadth is 39.6 for ♂ and 38.0 for ♀, indicating that in the present series the average palatal length is shorter by 6.9 mm. in ♂ and 4.1 mm. in ♀, while the average palatal breadth is 0.4 mm. greater in ♂ and 2.0 mm. shorter in ♀. The average palatal index is 93.7 for ♂ and 86.0 for ♀, whereas in Tildesley's Group A it is 79.8 in ♂ and 80.5 in ♀. There is thus a difference of 13.9 and 5.5 units respectively in the ♂ and ♀ between the two series. It is clear from this that the palates are decidedly broader in the present series than in Tildesley's Group A.

The facial profile angle shows that the skulls are either Mesognathous or Orthognathous, only 4 being Prognathous. The average facial profile angle is 83.5° in both the sexes. In Tildesley's Group A it is 86.0° for ♂ and 85.8° for ♀, showing that the present series is comparatively more prognathic than that of Tildesley.

Detailed measurements and statistical constants of the various linear dimensions and the angles of the Burmese crania are given in the following tables, Nos. XLII-XLV; the measurements of the facial projections are dealt with later.

TABLE XLII.—MEASUREMENTS OF BURMESE CRANIA (in mm.).

Number of the skull Sex	421 ♂	422 ♀	423 ♂	424 ♀	425 ♂	426 ♀	427 ♂	428 ♂	429 ♂
1. Maximum cranial length . . .	168	170	174	162	179	159	173	179	173
2. Maximum cranial breadth . . .	148	129	139	130	147	136	127	147	149
3. Nasion inion length . . .	163	157	169	156	165	155	154	173	167
4. Basilobregmatic height . . .	138	128	137	122	137	136	128	137	137
5. Least frontal breadth . . .	94	93	97	90	96	93	89	93	96
6. Greatest frontal breadth . . .	115	107	119	108	117	113	101	119	123
7. Bimastoid breadth . . .	112	98	107	89	106	97	97	—	103
8. Bizygomatic breadth . . .	—	120	145	—	—	128	123	—	139
9. Nasion basion line . . .	94	90	105	94	99	96	96	100	100
10. Prosthion basion line . . .	88	87	—	93	92	94	99	94	98
11. Nasion gnathion line . . .	*	—	*	—	122	110	*	—	—
12. Nasion prosthion line . . .	67	64	—	70	74	—	61	70	76
13. Nasal length . . .	47	47	57	51	56	48	48	51	55
14. Nasal breadth . . .	25	24	29	26	24	26	25	25	27
15. Interorbital breadth . . .	20	18	22	16	19	15	16	18	19
16. Orbital breadth—Right . . .	41	36	39	37	38	41	36	37	46
" " —Left . . .	39	35	39	36	39	41	36	39	46
17. Orbital height—Right . . .	35	32	35	35	31	34	29	33	38
" " —Left . . .	35	32	33	35	30	34	30	33	39
18. Maxilloalveolar breadth . . .	64	61	—	62	72	—	63	63	67
19. Maxilloalveolar length . . .	48	48	—	50	50	50	51	50	52
20. Palatal length . . .	—	45	—	42	46	45	43	44	46
21. Palatal breadth . . .	40	33	—	37	—	—	41	41	43
22. Occipital foramen—length . . .	34	33	38	30	34	34	37	38	36
Occipital foramen—breadth . . .	27	30	30	26	31	27	27	30	30
23. Sagittal cranial arc . . .	360	357	355	333	371	337	345	370	364
24. Transverse cranial arc . . .	311	279	315	282	308	296	273	315	316

TABLE XII.II.—MEASUREMENTS OF BURMESE CRANIA (contd.).

Number of the skull Sex	430 ♂	431 ♂	432 ♂	433 ♂	434 ♂	435 ♀	436 ♀	437 ♂	450 ♂
1. Maximum cranial length	169	180	172	185	181	170	167	160	186
2. Maximum cranial breadth	136	140	144	142	144	136	139	141	138
3. Nasioninion length	153	170	166	170	173	156	162	153	180
4. Basilobregmatic height	132	136	133	136	141	130	124	130	140
5. Least frontal breadth	93	81	91	98	96	94	90	93	93
6. Greatest frontal breadth	115	112	118	118	123	112	107	121	117
7. Bimastoid breadth	95	—	115	103	105	97	106	105	99
8. Bizygomatic breadth	128	133	131	—	135	123	128	126	132
9. Nasion basion line	94	99	99	100	102	96	96	92	105
10. Prosthion basion line	97	96	106	—	100	92	—	91	100
11. Nasion gnathion line	—	—	—	—	*	—	—	118	124
12. Nasion prosthion line	74	72	79	—	71	65	—	64	75
13. Nasal length	51	53	54	54	54	47	50	46	52
14. Nasal breadth	27	24	29	31	27	31	27	26	24
15. Interorbital breadth	18	16	16	19	23	23	18	18	12
16. Orbital breadth—Right	41	41	44	43	43	37	42	41	46
,, ,, —Left	41	38	42	41	41	38	43	39	46
17. Orbital height—Right	34	38	34	33	34	30	34	34	33
,, ,, —Left	34	38	34	34	33	31	34	35	33
18. Maxilloalveolar breadth	67	66	60	—	64	62	—	64	64
19. Maxilloalveolar length	55	52	56	—	65	50	—	47	57
20. Palatal length	44	44	48	—	49	40	—	33	46
21. Palatal breadth	43	38	41	39	37	34	—	38	37
22. Occipital foramen—length	39	—	35	35	32	32	34	35	36
Occipital foramen—breadth	31	—	29	29	30	27	27	29	29
23. Sagittal cranial arc	353	—	360	385	385	348	342	342	382
24. Transverse cranial arc	310	298	298	305	312	290	290	307	300

* Teeth lost.

TABLE XLII.—MEASUREMENTS OF BURMESE CRANIA (*contd.*).

Number of the skull Sex	451 ♂	452 ♂	453 ♂	454 ♂	455 ♂	456 ♂	457 ♂	459 ♂	460 ♂
1. Maximum cranial length . . .	172	174	157	173	165	167	178	177	183
2. Maximum cranial breadth . . .	142	137	146	143	141	136	141	134	133
3. Nasion inion length . . .	167	163	150	160	160	157	163	168	175
4. Basilobregmatic height . . .	135	131	135	131	133	131	130	142	140
5. Least frontal breadth . . .	87	91	86	99	87	92	95	99	93
6. Greatest frontal breadth . . .	116	115	120	120	109	116	121	119	110
7. Bimastoid breadth . . .	—	93	99	105	108	104	101	—	104
8. Bizygomatic breadth . . .	135	—	127	131	131	130	127	127	130
9. Nasion basion line . . .	93	100	92	99	97	90	95	101	108
10. Prosthion basion line . . .	96	96	—	94	—	93	88	93	106
11. Nasion gnathion line . . .	114	—	—	—	—	—	—	—	—
12. Nasion prosthion line . . .	68	65	—	70	—	65	67	68	74
13. Nasal length . . .	47	50	50	55	50	47	51	52	56
14. Nasal breadth . . .	28	—	24	25	29	30	25	24	26
15. Interorbital breadth . . .	21	—	21	20	19	15	21	18	20
16. Orbital breadth—Right . . .	42	42	40	45	42	45	39	43	41
" " —Left . . .	42	—	38	43	40	43	39	42	40
17. Orbital height—Right . . .	32	30	34	37	34	35	30	33	35
" " —Left . . .	33	—	35	36	34	37	29	33	34
18. Maxilloalveolar breadth . . .	71	61	64	70	—	67	67	61	64
19. Maxilloalveolar length . . .	54	50	48	54	—	54	52	52	58
20. Palatal length . . .	42	—	37	38	—	43	39	40	48
21. Palatal breadth . . .	42	—	36	40	—	39	40	39	39
22. Occipital foramen—length . . .	36	33	31	35	32	—	31	36	36
Occipital foramen—breadth . . .	26	29	26	30	29	—	26	30	30
23. Sagittal cranial arc . . .	361	356	347	343	350	—	375	364	361
24. Transverse cranial arc . . .	302	305	312	293	300	294	300	298	300

TABLE XLII.—MEASUREMENTS OF BURMESE CRANIA (*contd.*).

Number of the skull Sex	461 ♂	462 ♂	463 ♂	464 ♂	465 ♀	466 ♂	467 ♂	468 ♂	495 ♂
1. Maximum cranial length . . .	175	175	170	170	161	169	165	165	184
2. Maximum cranial breadth . . .	158	139	141	146	137	138	139	145	132
3. Nasioninion length . . .	161	157	154	159	158	160	157	155	172
4. Basilobregmatic height . . .	132	134	141	137	127	127	130	130	138
5. Least frontal breadth . . .	95	94	93	93	87	92	83	90	88
6. Greatest frontal breadth . . .	129	110	111	113	104	110	112	113	114
7. Bimastoid breadth . . .	100	111	101	106	104	91	108	100	102
8. Bizygomatic breadth . . .	—	137	131	135	124	121	126	135	135
9. Nasion basion line . . .	93	98	98	97	93	96	94	95	105
10. Prosthion basion line . . .	97	95	100	93	90	93	91	—	93
11. Nasion gnathion line . . .	—	—	—	—	—	—	—	—	—
12. Nasion prosthion line . . .	74	65	71	66	69	64	61	—	72
13. Nasal length . . .	52	51	47	49	48	49	48	52	51
14. Nasal breadth . . .	30	29	26	27	25	24	29	30	26.5
15. Interorbital breadth . . .	20	21	18	19	17	19	17	21	16
16. Orbital breadth—Right . . .	—	39	39	43	37	39	41	42	45
" " —Left . . .	44	39	40	42	38	39	40	41	42
17. Orbital height—Right . . .	—	33	33	33	33	29	32	33	34
" " —Left . . .	35	34	32	31	31	29	32	33	34
18. Maxilloalveolar breadth . . .	62	64	66	69	65	55	62	—	—
19. Maxilloalveolar length . . .	58	50	57	52	49	47	50	—	—
20. Palatal length . . .	43	46	45	43	41	42	45	—	39
21. Palatal breadth . . .	39	40	43	46	40	39	41	—	—
22. Occipital foramen—length . . .	36	37	36	33	32	32	35	35	34
Occipital foramen—breadth . . .	31	30	29	29	26	26	28	29	30
23. Sagittal cranial arc . . .	366	300	361	368	333	352	350	352	369
24. Transverse cranial arc . . .	327	290	304	305	285	290	294	294	298

TABLE XLII.—MEASUREMENTS OF BURMESE CRANIA (*contd.*).

Number of the skull Sex	421 ♂	422 ♀	423 ♂	424 ♀	425 ♂	426 ♀	427 ♂	428 ♂	429 ♂
25. Horizontal circum- ference . . .	505	480	515	476	513	471	479	515	512
26. Bicondylar breadth (mandible) . . .	—	—	124	—	—	111	115	—	—
27. Bigonial breadth . . .	—	—	105	—	102	104	84	—	—
28. (i) Length (height) of ramus . . .	—	—	60	—	60	56	63	—	—
(ii) Breadth of ramus :									
(a) Minimum . . .	28	—	34	—	38	30	30	—	—
(b) Maximum . . .	36	—	40	—	41	38	36	—	—
29. Symphyseal height . . .	22	—	28	—	31	32	28	—	—
30. Mandibular length . . .	—	—	72	—	74	67	70	—	—
31. Mandibular angle . . .	113°	—	119°	—	98°	119°	97°	—	—
32. Gnathion basion line . . .	—	—	119	—	101	101	104	—	—
33. Biauricular breadth . . .	127	113	131	114	127	118	117	127	127
34. Outer biorbital breadth . . .	106	97	110	97	107	103	95	104	110
35. Inner biorbital breadth . . .	100	90	102	90	96	95	90	96	105
36. Greatest occipital breadth . . .	110	100	115	95	109	104	106	114	110
37. Frontal arc . . .	130	125	127	119	125	120	115	130	135
38. Parietal arc . . .	125	127	124	119	130	116	117	110*	122
39. Occipital arc . . .	105	105	104	95	116	101	113	130*	107
40. Frontal chord . . .	114	107	113	103	111	107	101	114	118
41. Parietal chord . . .	115	111	110	104	113	102	106	—	117
42. Occipital chord . . .	91	91	92	82	98	89	92	—	90
43. Length of 1st Pre- molar to 3rd Molar . . .	—	—	—	—	—	—	—	40	—
44. Length of 1st Molar :									
Right { Antero- posterior . . .	—	10	—	9	11	—	10	10	—
Transverse . . .	—	11	—	11	10	—	10	11	—
Left { Antero- posterior . . .	10	10	—	—	10	—	—	8	—
Transverse . . .	11	11	—	—	11	—	—	10	—

TABLE XLII.—MEASUREMENTS OF BURMESE CRANIA (*contd.*).

Number of the skull Sex	430 ♂	431 ♂	432 ♂	433 ♂	434 ♂	435 ♀	436 ♀	437 ♂	450 ♂
25. Horizontal circum- ference . . .	490	509	506	523	520	489	490	485	523
26. Bicondylar breadth (mandible) . . .	—	—	—	—	—	—	—	110	119
27. Bigonial breadth . . .	—	—	—	—	94	—	—	93	92
28. (i) Length (height) of ramus . . .	—	—	—	—	—	—	—	56	64
(ii) Breadth of ramus :									
(a) Minimum . . .	—	—	—	—	35	—	—	31	33
(b) Maximum . . .	—	—	—	—	38	—	—	34	39
29. Symphyseal height . . .	—	—	—	—	—	—	—	34	34
30. Mandibular length . . .	—	—	—	—	68	—	—	64	74
31. Mandibular angle . . .	—	—	—	—	127°	—	—	117°	106°
32. Gnathion basion line . . .	—	—	—	—	—	—	—	101	109
33. Biauricular breadth . . .	120	124	129	122	126	121	125	120	124
34. Outer biorbital breadth . . .	101	94	102	106	109	101	102	101	106
35. Inner biorbital breadth . . .	93	90	95	99	102	94	97	94	98
36. Greatest occipital breadth . . .	108	106	114	107	104	105	108	109	100
37. Frontal arc . . .	128	127	116	140	135	126	115	126	133
38. Parietal arc . . .	120	120*	130	132	135*	116	115	114	137
39. Occipital arc . . .	105	—	114	113	115	106	112	102	112
40. Frontal chord . . .	109	111	104	121	117	110	106	111	119
41. Parietal chord . . .	105	—	109	116	—	105	103	100	118
42. Occipital chord . . .	93	—	101	94	—	91	95	90	93
43. Length of 1st Pre- molar to 3rd Molar . . .	43	—	43	—	—	—	—	—	—
44. Length of 1st Molar :									
Right { Antero- posterior . . .	11	11	11	—	12	9	—	10	—
{ Transverse . . .	12	13	11	—	12	10	—	10	—
Left { Antero- posterior . . .	11	11	11	—	12	—	—	10	11
{ Transverse . . .	12	12	11	—	11	—	—	10	10

* Wormian bones present.

TABLE XLII.—MEASUREMENTS OF BURMESE CRANIA (*contd.*).

Number of the skull Sex	451 ♂	452 ♂	453 ♂	454 ♂	455 ♂	456 ♂	457 ♂	459 ♂	460 ♂
25. Horizontal circumference .	499	499	478	505	491	491	509	498	512
26. Bicondylar breadth (mandible) .	—	—	—	—	—	—	—	—	—
27. Bigonial breadth .	96	92	—	—	—	—	—	—	—
28. (i) Length (height) of ramus .	—	—	—	—	—	—	—	—	—
(ii) Breadth of ramus :									
(a) Minimum .	34	35	—	—	—	—	—	—	—
(b) Maximum .	39	41	—	—	—	—	—	—	—
29. Symphyseal height .	31	32	—	—	—	—	—	—	—
30. Mandibular length .	67	73	—	—	—	—	—	—	—
31. Mandibular angle .	119°	120°	—	—	—	—	—	—	—
32. Gnathion basion line.	105	—	—	—	—	—	—	—	—
33. Biauricular breadth .	128	123	121	124	124	120	120	118	120
34. Outer biorbital breadth	104	102	99	109	98	101	104	105	109
35. Inner biorbital breadth	98	96	91	101	94	97	96	98	97
36. Greatest occipital breadth .	110	109	100	110	110	104	104	100	108
37. Frontal arc .	125	131	119	121	124	128	131	130	130
38. Parietal arc .	140*	125	130	111	111	136*	130	124	123
39. Occipital arc .	96*	100	98	111	115	—	114	110	108
40. Frontal chord .	111	112	107	110	113	111	112	113	116
41. Parietal chord .	—	111	106	101	99	—	114	110	112
42. Occipital chord .	—	86	89	96	98	—	96	93	94
43. Length of 1st Premolar to 3rd Molar	—	—	—	—	—	—	—	—	—
44. Length of 1st Molar :									
Right { Antero-posterior	11	—	10	10	—	9	9	10	9
{ Transverse .	11	—	10	10	—	11	10	11	11
Left { Antero-posterior	12	10	—	10	—	—	9	9	10
{ Transverse .	11	10	—	9	—	—	10	11	10

* Wormian bones present.

TABLE XLII.—MEASUREMENTS OF BURMESE CRANIA (*contd.*).

Number of the skull . Sex	461 ♂	462 ♂	463 ♂	464 ♂	465 ♀	466 ♂	467 ♂	468 ♂	495 ♂
25. Horizontal circum- ference . . .	528	502	493	508	478	495	490	493	522
26. Bicondylar breadth (mandible) . . .	—	—	—	—	—	—	—	—	—
27. Bigonial breadth . . .	—	—	—	—	—	—	—	—	—
28. (i) Length (height) of ramus . . .	—	—	—	—	—	—	—	—	—
(ii) Breadth of ramus :									
(a) Minimum . . .	—	—	—	—	—	—	—	—	—
(b) Maximum . . .	—	—	—	—	—	—	—	—	—
29. Symphyseal height . . .	—	—	—	—	—	—	—	—	—
30. Mandibular length . . .	—	—	—	—	—	—	—	—	—
31. Mandibular angle . . .	—	—	—	—	—	—	—	—	—
32. Gnathion basion line . . .	—	—	—	—	—	—	—	—	—
33. Biauricular breadth . . .	125	129	125	129	125	112	123	130	123
34. Outer biorbital breadth . . .	—	103	102	105	97	100	104	107	107
35. Inner biorbital breadth . . .	—	96	95	99	89	93	95	100	99
36. Greatest occipital breadth . . .	115	108	109	112	106	101	99	113	104
37. Frontal arc . . .	127	124	128	129	111	114	120	122	136
38. Parietal arc . . .	123	123	126	127*	117	123	120	121	123
39. Occipital arc . . .	116	113	107	102*	105	115	110	109	110
40. Frontal chord . . .	111	109	113	115	100	102	105	108	117
41. Parietal chord . . .	110	109	110	—	102	109	106	103	110
42. Occipital chord . . .	100	97	93	—	89	98	97	94	95
43. Length of 1st Pre- molar to 3rd Molar . . .	—	—	—	—	—	—	—	—	—
44. Length of 1st Molar :									
Right { Antero- posterior . . .	—	10	—	10	11	—	—	—	—
Transverse . . .	—	9	—	10	10	—	—	—	—
Left { Antero- posterior . . .	—	10	—	11	11	10	—	—	10
Transverse . . .	—	10	—	10	10	10	—	—	10

* Wormian bones present.

TABLE XLII.—MEASUREMENTS OF BURMESE CRANIA (*contd.*).

Number of the skull	421	422	423	424	425	426	427	428	429
Sex	♂	♀	♂	♀	♂	♀	♂	♂	♂
45. Length of 2nd Molar :									
Right { Antero-posterior	—	9	—	8	11	—	—	—	—
{ Transverse .	—	11	—	11	11	—	—	—	—
Left { Antero-posterior	—	—	—	—	—	—	—	9	—
{ Transverse .	—	—	—	—	—	—	—	10	—
46. Biorbito nasal arc .	107	97	110	96	102	100	95	103	113
47. Glabella nasion length .	16	15	10	13	7	12	14	11	11
48. Nasion lambda line .	—	163	170	158	174	154	162	—	168
49. Calvarial height .	—	101	103	93	109	98	104	103	107
50. Lambda calvarial height	—	70	71	65	73	70	64	—	76
51. Bregma position line .	—	93	98	87	99	93	93	99	104
52. Frontal perpendicular .	—	27	24	25	23	23	23	24	27
53. Parietal perpendicular .	—	26	19	24	28	24	20	—	21
54. Occipital perpendicular.	—	25	22	22	25	23	28	—	26
55. Frontal inclination angle	—	63°	61°	59°	62°	61°	66°	60°	62°
56. Occipital inclination angle	—	88°	82°	84°	87°	80°	83°	—	80°
57. Facial profile angle .	—	77°	—	84°	89°	81°	79°	86°	80°
58. Calvarial base angle .	—	16°	14°	10°	10°	—	10°	10°	17°
59. Frontal curvature angle	—	125°	134°	128°	138°	134°	131°	133°	131°
60. Parietal curvature angle	—	130°	134°	130°	132°	131°	138°	—	136°
61. Occipital curvature angle	—	123°	129°	125°	124°	129°	118°	—	119°
62. Occipital flexional angle	—	123°	129°	125°	123°	129°	117°	—	119°
63. Superior facial length angle .	—	42°	—	43°	44°	39°	36°	42°	44°
64. Nasion to foot of bregma perpendicular .	—	48	56	52	53	52	41	58	57
65. Cranial capacity in c.c. (calculated) .	1476	1226	1489	1204	1531	1286	1298	1560	1526

TABLE XLII.—MEASUREMENTS OF BURMESE CRANIA (*contd.*).

Number of the skull Sex	430 ♂	431 ♂	432 ♂	433 ♂	434 ♂	435 ♀	436 ♀	437 ♂	450 ♂
45. Length of 2nd Molar :									
Right { Antero- posterior	9	11	10	—	9	9	—	9	—
{ Transverse .	9	13	9	—	10	10	—	9	—
Left { Antero- posterior	8	—	10	—	10	9	—	8	10
{ Transverse .	9	—	10	—	9	9	—	10	10
46. Biobito nasal arc .	99	92	98	106	108	101	104	99	108
47. Glabella nasion length .	9	14	11	12	9	12	13	9	5
48. Nasion lambda line .	164	—	168	177	—	163	162	160	183
49. Calvarial height .	112	105	105	107	112	100	98	103	108
50. Lambda calvarial height	71	—	70	77	—	69	66	68	76
51. Bregma position line .	101	95	91	103	109	95	90	96	101
52. Frontal perpendicular .	27	26	20	29	29	25	20	28	25
53. Parietal perpendicular .	24	—	28	23	—	20	23	23	27
54. Occipital perpendicular.	17	—	26	26	—	22	23	20	28
55. Frontal inclination angle	68°	60°	63°	61°	61°	62°	59°	60°	59°
56. Occipital inclination angle	87°	84°	81°	85°	84°	86°	82°	86°	84°
57. Facial profile angle .	81°	80°	79°	87°	81°	86°	86°	84°	85°
58. Calvarial base angle .	15°	14°	12°	14°	12°	10°	12°	14°	11°
59. Frontal curvature angle	127°	129°	137°	129°	129°	130°	138°	128°	134°
60. Parietal curvature angle	133°	—	130°	136°	—	138°	132°	132°	131°
61. Occipital curvature angle	132°	—	126°	123°	—	127°	129°	132°	119°
62. Occipital flexional angle	132°	—	126°	124°	—	120°	123°	132°	119°
63. Superior facial length angle	45°	44°	44°	42°	40°	40°	—	42°	43°
64. Nasion to foot of bregma perpendicular . . .	43	54	48	60	57	53	54	54	61
65. Cranial capacity in c.c. (calculated) . . .	1391	1426	1489	1567	1549	1345	1341	1347	1521

TABLE XLII.—MEASUREMENTS OF BURMESE CRANIA (*contd.*).

Number of the skull Sex	451 ♂	452 ♂	453 ♂	454 ♂	455 ♂	456 ♂	457 ♂	459 ♂	460 ♂
45. Length of 2nd Molar :									
Right { Antero-posterior	10	*	—	9	—	—	9	10	10
Right { Transverse .	10	*	—	9	—	—	9	10	10
Left { Antero-posterior	10	8	—	9	—	—	9	10	10
Left { Transverse .	9	7.5	—	7	—	—	11	11	11
46. Biorbito nasal arc .	104	104	97	110	103	101	103	107	105
47. Glabella nasion length .	12	—	18	11	11	16	9	10	9
48. Nasion lambda line .	—	168	155	168	162	—	174	166	178
49. Calvarial height . .	104	103	100	100	103	105	108	103	98
50. Lambda calvarial height	—	74	71	61	67	—	73	75	70
51. Bregma position line .	97	98	92	93	97	100	101	99	95
52. Frontal perpendicular .	25	27	23	22	22	26	31	27	25
53. Parietal perpendicular .	—	24	27	18	18	—	23	22	21
54. Occipital perpendicular .	—	23	19	23	26	—	25	28	25
55. Frontal inclination angle	61°	62°	61°	57°	59°	65°	63°	63°	55°
56. Occipital inclination angle	—	87°	87°	85°	82°	—	90°	80°	86°
57. Facial profile angle .	83°	87°	87°	82°	87°	78°	86°	81°	81°
58. Calvarial base angle .	—	10°	—	13°	11°	14°	17°	14°	—
59. Frontal curvature angle	133°	128°	131°	135°	137°	128°	124°	130°	134°
60. Parietal curvature angle	—	133°	127°	138°	140°	—	137°	138°	139°
61. Occipital curvature angle	—	126°	134°	125°	124°	—	122°	123°	126°
62. Occipital flexional angle	—	126°	134°	125°	124°	—	122°	123°	126°
63. Superior facial length angle . . .	41°	38°	45°	42°	38°	41°	43°	40°	41°
64. Nasion to foot of bregma perpendicular . .	52	53	50	59	56	47	49	52	64
65. Cranial capacity in c.c. (calculated) . .	1474	1438	1388	1389	1395	1346	1440	1415	1470

TABLE XLII.—MEASUREMENTS OF BURMESE CRANIA (*contd.*).

Number of the skull Sex	461 ♂	462 ♂	463 ♂	464 ♂	465 ♀	466 ♂	467 ♂	468 ♂	495 ♂
45. Length of 2nd Molar :									
Right { Antero-posterior	—	10	—	10	9	—	—	—	—
Right { Transverse	—	10	—	8	10	—	—	—	—
Left { Antero-posterior	9	9	—	9	10	9	10	—	8
Left { Transverse	11	10	—	9	9	9	9	—	9
46. Biorbito nasal arc	—	102	100	106	94	97	99	107	103
47. Glabella nasion length	13	11	14	12	13	11	12	12	11
48. Nasion lambda line	169	169	163	—	155	166	158	161	178
49. Calvarial height	106	110	114	114	90	107	99	109	103
50. Lambda calvarial height	71	67	74	—	65	66	67	68	72
51. Bregma position line	99	98	103	104	83	91	92	95	99
52. Frontal perpendicular	26	26	27	24	21	21	25	23	29
53. Parietal perpendicular	22	24	26	—	23	23	24	26	22
54. Occipital perpendicular	24	22	20	—	25	25	24	23	27
55. Frontal inclination angle	63°	64°	68°	65°	57°	63°	62°	63°	56°
56. Occipital inclination angle	87°	88°	84°	—	79°	84°	82°	84°	86°
57. Facial profile angle	85°	84°	82°	87°	87°	83°	80°	—	89°
58. Calvarial base angle	—	14°	13°	14°	17°	14°	13°	15°	11°
59. Frontal curvature angle	131°	129°	129°	134°	135°	136°	130°	133°	127°
60. Parietal curvature angle	135°	132°	128°	—	132°	135°	130°	128°	136°
61. Occipital curvature angle	125°	131°	130°	—	123°	124°	126°	121°	123°
62. Occipital flexional angle	125°	128°	127°	—	123°	124°	126°	121°	123°
63. Superior facial length angle	44°	39°	39°	41°	43°	40°	37°	—	41°
64. Nasion to foot of bregma perpendicular	50	44	42	47	54	44	50	44	64
65. Cranial capacity in c.c. (calculated)	1661	1425	1461	1501	1272	1338	1322	1372	1441

After the detailed Measurements of the Burmese Crania their Indices are given in the series of Table XLIII.

TABLE XLIII.—INDICES OF BURMESE CRANIA.

Number of the skull Sex	421 ♂	422 ♀	423 ♂	424 ♀	425 ♂	426 ♀	427 ♂	428 ♂	429 ♂
<i>Indices of the Cranium :</i>									
1. Length breadth index .	88.1	75.9	79.9	80.2	82.1	84.9	73.4	82.1	80.3
2. Length height index .	78.6	75.3	78.7	75.3	76.5	85.5	74.0	76.5	79.2
3. Breadth height index .	89.2	99.2	98.6	93.8	93.2	100.0	100.8	93.2	91.9
4. Calvarial height index .	—	64.3	60.9	59.6	66.1	63.2	67.5	59.5	64.1
5. Bregma position index .	—	30.6	33.1	34.6	32.1	33.5	26.6	33.5	34.1
6. Sagittal cranial curva- ture index .	45.3	44.0	47.6	46.8	44.5	46.0	44.6	47.0	45.9
7. Transverse cranial curva- ture index .	40.8	40.5	41.6	40.4	41.2	39.8	42.8	40.3	40.2
8. Transverse fronto parie- tal index .	63.5	72.1	69.8	69.2	65.3	68.4	70.1	63.3	65.1
9. <i>Indices showing the rela- tions of the various sagittal arcs :</i>									
(a) Fronto parietal index .	96.1	101.6	97.6	100.0	104.0	96.7	101.7	84.6	90.3
(b) Fronto occipital index .	80.8	84.0	81.9	79.8	92.8	84.2	98.2	100.0	79.3
(c) Parieto occipital index .	84.0	82.7	83.9	79.8	89.2	87.1	96.6	118.2	87.7
(d) Fronto sagittal arc index .	36.1	35.0	35.8	35.7	33.7	35.6	33.3	35.1	37.1
(e) Parieto sagittal arc index .	34.7	35.6	35.0	35.7	35.0	34.4	33.9	29.7	33.5
(f) Occipito sagittal arc index .	29.2	29.4	29.3	28.5	31.2	26.8	32.7	37.1	29.4
10. <i>Indices showing the amount of curvature (bulging) of each of the three contour bones of the cranium :</i>									
(a) Frontal curvature index .	87.7	85.6	96.0	86.6	88.8	89.2	87.8	87.7	87.4
(b) Parietal curvature index .	92.0	87.4	88.7	87.4	86.9	87.9	90.6	—	95.9
(c) Occipital curvature index .	86.7	86.7	88.5	86.3	84.5	88.1	81.4	—	84.1
<i>Indices of the Face :</i>									
11. Total facial index .	—	—	—	—	—	85.9	—	—	—
12. Superior facial index .	—	53.3	—	—	—	—	49.6	—	54.7
13. Zygomatico frontal index	—	77.5	66.9	—	—	72.7	72.4	—	69.1
14. Zygomatico mandibular index .	—	—	72.4	—	—	81.2	68.3	—	—
15. Interorbital index .	18.9	18.6	20.0	16.5	17.7	14.6	16.8	17.3	17.2

TABLE XLIII.—INDICES OF BURMESE CRANIA (*contd.*).

Number of the skull Sex	430 ♂	431 ♂	432 ♂	433 ♂	434 ♂	435 ♀	436 ♀	437 ♂	450 ♂
<i>Indices of the Cranium :</i>									
1. Length breadth index . . .	80.5	77.7	83.7	76.8	79.6	80.0	83.2	88.1	74.2
2. Length height index . . .	78.1	75.6	77.3	73.5	77.9	76.5	76.0	81.2	75.3
3. Breadth height index . . .	97.1	97.1	92.4	95.8	97.2	95.6	91.4	92.2	101.4
4. Calvarial height index . . .	73.2	61.8	63.2	62.9	64.7	64.1	60.5	67.3	60.0
5. Bregma position index . . .	28.0	31.7	28.9	35.3	32.9	34.0	33.3	35.3	33.9
6. Sagittal cranial curvature index . . .	43.3	—	46.1	44.1	44.9	44.8	47.3	44.7	47.1
7. Transverse cranial curvature index . . .	38.7	41.6	43.3	40.0	40.3	41.7	43.1	39.1	41.3
8. Transverse frontal parietal index . . .	68.4	57.8	63.2	69.0	66.7	69.1	64.7	66.0	67.4
9. <i>Indices showing the relations of the various sagittal arcs :</i>									
(a) Fronto parietal index . . .	93.7	94.5	112.1	94.3	100.0	92.1	100.0	90.5	103.0
(b) Fronto occipital index . . .	82.0	—	98.3	80.7	85.2	84.1	97.4	80.9	84.2
(c) Parieto occipital index . . .	87.5	—	87.7	85.6	85.2	91.4	97.6	89.5	81.8
(d) Fronto sagittal arc index . . .	36.3	—	32.2	36.4	35.1	36.2	33.6	36.8	34.8
(e) Parieto sagittal arc index . . .	34.0	—	36.1	34.3	35.1	33.3	33.6	33.3	35.9
(f) Occipito sagittal arc index . . .	29.7	—	31.7	29.3	29.9	30.5	32.7	29.8	29.3
10. <i>Indices showing the amount of curvature (bulging) of each of the three contour bones of the cranium :</i>									
(a) Frontal curvature index . . .	85.2	87.4	89.7	86.4	86.7	87.3	92.2	88.1	89.5
(b) Parietal curvature index . . .	87.5	—	83.8	87.9	—	90.5	89.5	87.7	86.1
(c) Occipital curvature index . . .	88.6	—	88.6	83.2	—	85.8	84.8	88.2	83.0
<i>Indices of the Face :</i>									
11. Total facial index . . .	—	—	—	—	—	—	—	93.6	93.9
12. Superior facial index . . .	57.8	54.1	60.3	—	52.6	52.8	—	50.8	56.8
13. Zygomatico frontal index . . .	72.7	60.9	69.5	—	71.1	76.4	70.3	73.8	70.4
14. Zygomatico mandibular index . . .	—	—	—	—	69.6	—	—	73.8	69.7
15. Interorbital index . . .	17.8	17.0	15.7	17.9	21.1	22.8	17.6	17.8	11.3

TABLE XLIII.—INDICES OF BURMESE CRANIA (*contd.*).

Number of the skull Sex	451 ♂	452 ♂	453 ♂	454 ♂	455 ♂	456 ♂	457 ♂	459 ♂	460 ♂
<i>Indices of the Cranium :</i>									
1. Length breadth index .	82.6	78.7	93.0	82.7	85.5	81.4	79.2	75.7	72.7
2. Length height index .	78.5	75.3	86.0	75.7	80.6	78.4	73.0	80.2	76.5
3. Breadth height index .	95.1	95.6	92.5	91.6	94.3	96.3	90.2	106.0	105.3
4. Calvarial height index .	62.3	63.2	66.7	62.5	64.4	66.9	66.3	61.3	52.0
5. Bregma position index .	31.1	32.5	33.3	36.9	35.0	29.9	30.1	30.9	36.6
6. Sagittal cranial curvature index .	46.2	45.8	43.2	46.6	45.7	—	43.5	46.1	48.4
7. Transverse cranial curvature index .	42.4	40.3	38.8	42.3	41.3	40.8	40.0	39.6	40.0
8. Transverse fronto parietal index .	61.3	66.4	58.9	69.2	61.7	67.6	67.4	73.9	69.9
<i>9. Indices showing the relations of the various sagittal arcs :</i>									
(a) Fronto parietal index .	112.0	95.4	109.2	91.7	89.5	106.2	99.2	95.4	94.6
(b) Fronto occipital index .	76.8	76.3	82.3	91.7	92.7	—	87.0	84.6	83.1
(c) Parieto occipital index .	68.6	80.0	75.4	100.0	103.6	—	87.7	88.7	87.8
(d) Fronto sagittal arc index .	34.6	36.8	34.3	35.3	35.4	—	34.9	35.7	36.0
(e) Parieto sagittal arc index .	38.8	35.1	37.4	32.3	31.7	—	34.6	34.1	34.1
(f) Occipito sagittal arc index .	26.6	28.1	28.2	32.3	32.8	—	30.4	30.2	29.9
<i>10. Indices showing the amount of curvature (bulging) of each of the three contour bones of the cranium :</i>									
(a) Frontal curvature index .	88.8	85.5	89.9	90.9	91.9	—	85.5	86.9	89.2
(b) Parietal curvature index .	—	88.8	81.5	91.0	89.2	—	—	88.7	91.1
(c) Occipital curvature index .	—	86.0	90.8	86.5	85.2	—	—	84.5	87.0
<i>Indices of the Face :</i>									
11. Total facial index .	84.4	—	—	—	—	—	—	—	—
12. Superior facial index .	50.4	—	—	53.4	—	50.0	52.8	53.5	56.9
13. Zygomatico frontal index	64.4	—	67.7	75.6	66.4	70.8	74.8	77.9	71.5
14. Zygomatico mandibular index .	71.1	—	—	—	—	—	—	—	—
15. Interorbital index .	20.2	—	21.2	18.3	19.4	14.8	20.2	17.1	18.3

TABLE XLIII.—INDICES OF BURMESE CRANIA (*contd.*).

Number of the skull Sex	461 ♂	462 ♂	463 ♂	464 ♂	465 ♀	466 ♂	467 ♂	468 ♂	495 ♂
<i>Indices of the Cranium :</i>									
1. Length breadth index .	90.3	79.4	82.9	85.9	85.1	81.7	84.2	87.9	71.7
2. Length height index .	75.4	76.6	82.9	80.6	78.9	75.1	78.8	78.8	75.0
3. Breadth height index .	83.5	96.4	100.0	93.8	92.7	92.0	93.5	89.7	104.5
4. Calvarial height index .	65.8	70.1	74.0	71.7	57.0	66.9	63.1	70.3	59.9
5. Bregma position index .	31.0	28.0	27.3	29.5	34.2	27.5	31.8	28.4	37.2
6. Sagittal cranial curva- ture index . . .	44.0	52.3	42.6	43.2	47.4	45.4	44.9	44.0	46.6
7. Transverse cranial curva- ture index . . .	38.2	44.5	41.1	42.3	43.8	38.6	41.8	44.2	41.3
8. Transverse fronto parie- tal index . . .	60.1	67.6	66.0	67.3	63.5	66.7	59.7	62.1	66.7
9. <i>Indices showing the rela- tions of the various sagittal arcs :</i>									
(a) Fronto parietal index . . .	96.8	99.2	98.4	106.2	105.4	107.9	100.0	99.2	90.4
(b) Fronto occipital index . . .	90.6	91.1	83.6	79.0	94.6	100.9	91.7	89.3	80.9
(c) Parieto occipital index . . .	94.3	91.8	84.9	74.4	89.8	93.5	91.7	90.1	89.4
(d) Fronto sagittal arc index . . .	34.7	41.3	35.2	35.1	33.3	32.3	34.3	34.6	36.8
(e) Parieto sagittal arc index . . .	33.6	41.0	34.9	37.2	35.1	34.9	34.3	34.3	33.3
(f) Occipito sagittal arc index . . .	31.8	37.6	29.6	27.7	31.5	32.7	31.4	30.9	29.8
10. <i>Indices showing the amount of curvature (bulging) of each of the three contour bones of the cranium :</i>									
(a) Frontal curvature index . . .	87.4	87.9	88.3	89.1	90.1	89.4	87.5	88.5	86.0
(b) Parietal curvature index . . .	89.4	88.6	87.3	—	87.2	88.6	88.3	85.1	89.4
(c) Occipital curvature index . . .	86.2	85.8	86.9	—	84.8	85.2	88.2	86.2	86.4
<i>Indices of the Face :</i>									
11. Total facial index .	—	—	—	—	—	—	—	—	—
12. Superior facial index .	—	47.0	54.2	48.1	55.6	52.9	48.4	—	53.3
13. Zygomatico frontal index	—	68.6	71.0	68.9	70.2	76.0	65.9	66.7	65.2
14. Zygomatico mandibular index . . .	—	—	—	—	—	—	—	—	—
15. Interorbital index .	—	20.4	17.6	18.1	17.5	19.0	16.3	19.6	14.9

TABLE XLIII.—INDICES OF BURMESE CRANIA (*contd.*).

Number of the skull Sex	421 ♂	422 ♀	423 ♂	424 ♀	425 ♂	426 ♀	427 ♂	428 ♂	429 ♂
16. Orbital index—Right .	85.4	88.9	89.7	94.6	81.6	82.9	80.6	89.2	82.6
" " —Left .	89.7	91.1	84.6	97.2	76.9	82.9	83.3	84.6	84.8
17. Nasal index .	53.2	51.1	50.9	51.0	42.9	54.2	52.1	49.0	49.1
18. Maxillo alveolar index .	133.3	127.1	—	124.0	144.0	—	123.5	126.0	128.9
19. Palatal index .	—	73.3	—	88.1	—	—	95.3	93.2	93.5
20. Mandibular index .	—	—	58.1	—	—	60.4	60.9	—	—
21. Ramus index .	—	—	56.7	—	63.3	53.6	47.6	—	—
22. Dental index .	—	—	—	—	—	—	—	40.0	—
<i>Indices showing relations between cranium and face :</i>									
23. Longitudinal cranio facial index .	52.4	51.2	—	57.4	51.4	59.1	57.2	52.5	56.6
24. Transverse cranio facial index .	—	93.0	104.3	—	—	94.1	96.8	—	93.3
25. Vertical cranio facial index .	50.8	50.0	—	57.4	54.1	—	47.7	51.1	56.9
<i>Some additional indices :</i>									
26. Lambda calvarial height index .	—	42.9	41.8	41.1	41.9	45.4	39.5	—	44.6
27. Frontal perpendicular index .	—	25.2	21.2	24.3	20.7	21.5	22.8	21.0	22.9
28. Parietal perpendicular index .	—	23.4	17.3	23.1	24.8	23.5	18.9	—	17.9
29. Occipital perpendicular index .	—	27.5	23.9	26.8	25.5	25.8	30.4	—	28.9
30. Meatal position index .	52.8	55.4	56.2	58.4	55.2	55.6	55.2	56.1	56.3

TABLE XLIII.—INDICES OF BURMESE CRANIA (*contd.*).

Number of the skull Sex	430 ♂	431 ♂	432 ♂	433 ♂	434 ♂	435 ♀	436 ♀	437 ♂	438 ♂
16. Orbital index—Right .	82.9	92.7	77.3	76.7	79.1	81.1	80.9	82.9	71.7
" " —Left .	82.9	100.0	80.9	82.9	80.5	81.6	79.1	89.7	71.7
17. Nasal index .	54.9	45.3	53.7	57.4	50.0	66.0	54.0	56.5	46.1
18. Maxillo alveolar index .	121.8	126.9	107.1	—	98.4	124.0	—	136.1	112.3
19. Palatal index .	97.7	86.4	85.4	—	75.5	85.0	—	115.1	80.4
20. Mandibular index .	—	—	—	—	—	—	—	58.2	62.2
21. Ramus index .	—	—	—	—	—	—	—	55.4	51.6
22. Dental index .	45.7	—	43.4	—	—	—	—	—	—
<i>Indices showing relations between cranium and face :</i>									
23. Longitudinal cranio facial index .	57.4	53.3	61.6	—	55.2	54.1	—	56.9	53.8
24. Transverse cranio facial index .	94.1	95.0	91.0	—	93.7	90.4	92.1	89.4	95.6
25. Vertical cranio facial index .	56.1	52.9	59.4	—	50.3	50.0	—	49.2	53.6
<i>Some additional indices :</i>									
26. Lambda calvarial height index .	42.6	—	41.7	43.5	—	42.3	40.7	42.5	41.5
27. Frontal perpendicular index .	24.8	23.4	19.2	24.0	24.8	22.7	18.9	25.2	21.0
28. Parietal perpendicular index .	22.9	—	25.7	19.8	—	19.0	22.3	23.0	22.9
29. Occipital perpendicular index .	18.3	—	25.7	27.7	—	24.2	24.2	22.2	30.1
30. Meatal position index .	60.0	50.0	54.2	55.9	54.9	55.2	54.4	58.2	52.8

TABLE XLIII.—INDICES OF BURMESE CRANIA (*contd.*).

Number of the skull Sex	451 ♂	452 ♂	453 ♂	454 ♂	455 ♂	456 ♂	457 ♂	459 ♂	460 ♂
16. Orbital index—Right .	76.2	71.4	85.0	82.2	80.9	77.8	76.9	76.7	85.4
" " —Left .	78.6	—	92.1	83.7	85.0	86.0	74.3	78.6	85.0
17. Nasal index . . .	59.6	—	48.0	45.4	58.0	63.8	49.0	46.1	46.4
18. Maxillo alveolar index .	131.5	122.0	133.3	129.6	—	124.1	128.9	117.3	110.3
19. Palatal index . . .	100.0	—	97.3	105.3	—	90.7	102.6	97.5	81.2
20. Mandibular index .	—	—	—	—	—	—	—	—	—
21. Ramus index . . .	—	56.4	—	—	—	—	—	—	—
22. Dental index . . .	—	—	—	—	—	—	44.2	—	—
<i>Indices showing relations between cranium and face :</i>									
23. Longitudinal cranio facial index	55.8	55.2	—	54.3	—	55.7	49.4	52.5	57.9
24. Transverse cranio facial index	95.1	—	87.0	91.6	92.9	95.6	90.1	94.8	97.7
25. Vertical cranio facial index	50.4	49.6	—	53.4	—	49.6	51.4	47.9	52.9
<i>Some additional indices :</i>									
26. Lambda calvarial height index	—	44.0	45.8	36.3	41.3	—	41.9	45.2	39.3
27. Frontal perpendicular index	22.5	24.1	21.7	20.0	19.5	23.4	27.7	23.9	21.5
28. Parietal perpendicular index	—	21.6	25.5	17.8	18.2	—	20.2	22.0	18.7
29. Occipital perpendicular index	—	26.7	21.3	24.0	26.5	—	26.0	30.1	26.6
30. Meatal position index .	52.1	58.3	54.7	55.0	52.5	54.8	57.1	51.2	53.8

TABLE XLIII.—INDICES OF BURMESE CRANIA (*continued*).

Number of the skull Sex	461 ♂	462 ♂	463 ♂	464 ♂	465 ♀	466 ♂	467 ♂	468 ♂	495 ♂
16. Orbital index—Right .	—	84.6	84.6	76.7	89.2	74.3	78.0	78.6	75.6
„ „ —Left .	79.5	87.2	80.0	73.8	81.6	74.3	80.0	80.5	80.9
17. Nasal index . . .	57.7	56.9	55.3	55.1	52.1	49.0	60.4	57.7	51.1
18. Maxillo alveolar index .	106.9	128.0	115.8	113.4	132.6	117.0	124.0	—	—
19. Palatal index . . .	90.7	87.0	95.5	107.0	97.6	92.9	91.1	—	—
20. Mandibular index .	—	—	—	—	—	—	—	—	—
21. Ramus index . . .	—	—	—	—	—	—	—	—	—
22. Dental index . . .	—	—	—	—	—	—	—	—	—
<i>Indices showing relations between cranium and face :</i>									
23. Longitudinal cranio facial index	55.4	54.3	59.2	54.7	55.9	55.0	55.1	—	50.5
24. Transverse cranio facial index	—	98.6	92.9	92.5	90.5	87.7	90.6	93.1	102.3
25. Vertical cranio facial index	56.1	48.5	50.3	48.2	54.3	50.4	46.9	—	52.2
<i>Some additional indices :</i>									
26. Lambda calvarial height index	42.0	39.6	45.4	—	41.9	39.7	42.4	42.2	40.4
27. Frontal perpendicular index	23.4	23.8	23.9	20.9	21.0	20.6	23.8	21.3	24.8
28. Parietal perpendicular index	22.0	22.0	23.6	—	22.5	21.0	22.6	25.2	20.0
29. Occipital perpendicular index	24.0	22.7	21.5	—	28.1	25.5	24.7	24.5	28.4
30. Meatal position index .	54.0	58.6	60.4	57.3	55.0	60.6	54.2	55.5	55.8

TABLE XLIV.—BURMESE CRANIA—*Statistical Constants.*
Indices (MALE).

	No.	Mean	Standard Deviation	Coefficient of Variation
<i>Indices of the Cranium :</i>				
1. Length breadth index	30	81.4 ± 0.62	5.09 ± 0.44	6.25 ± 0.54
2. Length height index	30	77.6 ± 0.34	2.81 ± 0.24	3.62 ± 0.31
3. Breadth height index	30	95.3 ± 0.60	4.88 ± 0.43	5.12 ± 0.43
4. Calvarial height index	29	64.8 ± 0.56	4.51 ± 0.40	6.93 ± 0.61
5. Bregma position index	29	31.8 ± 0.37	2.99 ± 0.26	9.40 ± 0.83
6. Sagittal cranial curvature index	28	45.5 ± 0.24	1.93 ± 0.17	4.24 ± 0.38
7. Transverse cranial curvature index	30	40.9 ± 0.19	1.55 ± 0.13	3.79 ± 0.28
8. Transverse fronto parietal index	30	65.6 ± 0.45	3.71 ± 0.32	5.65 ± 0.49
9. <i>Indices showing the relations of the various sagittal arcs :</i>				
(a) Fronto parietal index	30	98.4 ± 0.78	6.37 ± 0.55	6.47 ± 0.58
(b) Fronto occipital index	28	86.6 ± 0.89	6.98 ± 0.63	8.06 ± 0.74
(c) Parieto occipital index	28	88.5 ± 1.16	9.16 ± 0.82	10.35 ± 0.93
(d) Fronto sagittal arc index	28	35.3 ± 0.21	1.67 ± 0.15	4.73 ± 0.42
(e) Parieto sagittal arc index	28	34.7 ± 0.26	2.09 ± 0.19	6.02 ± 0.54
(f) Occipito sagittal arc index	28	30.7 ± 0.30	2.40 ± 0.21	7.81 ± 0.70
10. <i>Indices showing the amount of curvature (bulging) of each of the three contour bones of the cranium :</i>				
(a) Frontal curvature index	29	88.3 ± 0.26	2.12 ± 0.18	2.40 ± 0.21
(b) Parietal curvature index	23	88.4 ± 0.40	2.84 ± 0.28	3.21 ± 0.32
(c) Occipital curvature index	23	86.2 ± 0.29	2.10 ± 0.21	2.43 ± 0.24
<i>Indices of the Face :</i>				
11. Total facial index	3	90.6	—	—
12. Superior facial index	20	52.9 ± 0.50	3.35 ± 0.35	6.33 ± 0.67

TABLE XLIV.—BURMESE CRANIA—*Statistical Constants.*
Indices (MALE) (continued).

	No.	Mean	Standard Deviation	Coefficient of Variation
13. Zygomatico frontal index .	24	69.9 ± 0.55	3.99 ± 0.39	5.70 ± 0.55
14. Zygomatico mandibular index	6	70.8	—	—
15. Interorbital index . .	28	17.9 ± 0.27	2.10 ± 0.19	11.73 ± 1.07
16. Orbital index—Right . .	29	80.6 ± 0.64	5.11 ± 0.45	6.46 ± 0.57
" " —Left . .	29	82.5 ± 0.73	5.83 ± 0.51	7.06 ± 0.62
17. Nasal index . . .	29	52.5 ± 0.66	5.30 ± 0.47	10.09 ± 0.89
18. Maxillo alveolar index .	25	122.4 ± 1.38	10.23 ± 0.97	8.35 ± 0.80
19. Palatal index . . .	22	93.4 ± 1.30	9.03 ± 0.92	9.66 ± 0.98
20. Mandibular index . . .	4	59.8	—	—
21. Ramus index . . .	6	55.2	—	—
22. Dental index . . .	4	43.3	—	—
<i>Indices showing relations between cranium and face :</i>				
23. Longitudinal cranio facial index	25	54.9 ± 0.36	2.66 ± 0.25	4.84 ± 0.65
24. Transverse cranio facial index	24	94.0 ± 0.55	4.00 ± 0.39	4.25 ± 0.41
25. Vertical cranio facial index .	25	51.6 ± 0.41	3.09 ± 0.29	6.00 ± 0.57
<i>Some additional indices :</i>				
26. Lambda calvarial height index	23	42.0 ± 0.33	2.38 ± 0.23	5.66 ± 0.56
27. Frontal perpendicular index.	29	22.7 ± 0.24	1.93 ± 0.17	8.50 ± 0.75
28. Parietal perpendicular index	23	21.5 ± 0.35	2.53 ± 0.25	11.76 ± 1.19
29. Occipital perpendicular index	23	25.4 ± 0.42	3.01 ± 0.30	11.85 ± 1.19
30. Meatal position index . .	30	55.5 ± 0.31	2.56 ± 0.22	4.61 ± 0.40

In Table XLV are given the average indices of Burmese Female Crania.

TABLE XLV.—BURMESE CRANIA—*Average Indices of Burmese Crania (FEMALES).*

	No.	Average
<i>Indices of the Cranium :</i>		
1. Length breadth index	6	81·1
2. Length height index	6	77·9
3. Breadth height index	6	95·5
4. Calvarial height index	6	61·5
5. Bregma position index	6	33·4
6. Sagittal cranial curvature index	6	46·5
7. Transverse cranial curvature index	6	41·5
8. Transverse fronto parietal index	6	67·8
9. <i>Indices showing the relations of the various sagittal arcs :</i>		
(a) Fronto parietal index	6	99·3
(b) Fronto occipital index	6	87·3
(c) Parieto occipital index	6	88·7
(d) Fronto sagittal arc index	6	34·9
(e) Parieto sagittal arc index	6	34·6
(f) Occipito sagittal arc index	6	29·9
10. <i>Indices showing the amount of curvature (bulging) of each of the three contour bones of the cranium :</i>		
(a) Frontal curvature index	6	88·5
(b) Parietal curvature index	6	88·3
(c) Occipital curvature index	6	86·1
<i>Indices of the Face :</i>		
11. Total facial index	1	85·9
12. Superior facial index	3	53·9
13. Zygomatico frontal index	5	73·4
14. Zygomatico mandibular index	1	81·2
15. Interorbital index	6	17·9

TABLE XLV.—BURMESE CRANIA—*Average Indices of Burmese Crania (FEMALES) (continued).*

	No.	Average
16. Orbital index—Right	6	86.3
„ „ —Left	6	85.6
17. Nasal index	6	54.7
18. Maxillo alveolar index	4	126.9
19. Palatal index	4	86.0
20. Mandibular index	1	60.4
21. Ramus index	1	53.6
22. Dental index	—	—
<i>Indices showing relations between cranium and face :</i>		
23. Longitudinal cranio facial index	5	55.5
24. Transverse cranio facial index	5	92.0
25. Vertical cranio facial index	4	52.9
<i>Some additional indices :</i>		
26. Lambda calvarial height index	6	42.4
27. Frontal perpendicular index	6	22.3
28. Parietal perpendicular index	6	22.3
29. Occipital perpendicular index	6	26.1
30. Meatal position index	6	55.8

THE FACIAL PROJECTIONS

The importance of the facial cast in the study of racial differences is very great. Recently Sir Arthur Keith¹ has

¹ Sir Arthur Keith, 'Reports on the Human Remains' (pp. 231-239) in *Ur Excavations*, vol. i (Al-U-Baid), by H. R. Hall and C. L. Woolley (Oxford, 1927). See also *idem.*, 'Human Skulls from Ancient Tarim Basin,' *Journ. Roy. Anthropol. Inst.*, vol. lix, pp. 168-171 (London, 1929).

devised a method for the study of the degree of the forward growth in the component elements of the face by projections drawn in an antero-posterior plane. The measurements of Burmese crania, taken according to Keith's method, are given with their averages in Tables XLVI-XLIX.

The average glabellar projection from the mid-meatal plane is 88.5 mm. for ♂ and 85.5 for ♀, and the average projection of the nasion is 85.8 mm. for ♂ and 82.8 for ♀. The difference between the two is 2.7 mm. for ♂ and 3.0 for ♀, which gives the depth of the sub-glabellar notch at the root of the nose. In the Chinese skulls measured by Sir Arthur Keith¹ it is 5.5 mm., and in the Naga Group I it is 2.1 mm.²

Coming to the projection of the nasion in advance of the lateral orbital point, we find it to be 16.4 mm. for ♂ and 12.8 for ♀.

The difference between the projection of the lateral orbital point and the tip of the nose from the mid-meatal plane gives us the amount of the forward projection of the tip of the nose from the lateral orbital point. This is 23.3 mm. for ♂ and 18.0 mm. for ♀. In the Chinese skulls it is 21.5 mm., and in the Naga Group I it is 14.9 mm.

The height of the most advanced part of the dorsum of the nose from the inferior orbital margin gives us another means of measuring the differences in the projection of the nose. This difference is 16.8 mm. for ♂ and 15.0 mm. for ♀. In the Chinese skulls it is 14.5 mm., and in the Naga Group I it is 16.5 mm.

The projection of the lower malomaxillary point in front of the mid-meatal plane is 66.8 mm. for ♂ and 65.0 mm. for ♀. The difference between the projection of the lower malomaxillary point and the projection of the lateral orbital point is 2.6 mm. for ♂ and 5.0 mm. for ♀. In Naga Group I it is only 1.0 mm.

The projection of the least advanced part of the nose from the mid-meatal plane is 82.7 mm. for ♂ and 79.0 for ♀. The difference between it and the lower malomaxillary point

¹ Sir Arthur Keith (*loc. cit.*), *Journ. Roy. Anthropol. Inst.*, vol. lix, p. 170.

² B. S. Guha and P. C. Basu, *loc. cit.*, p. 15.

is 15.9 mm. for ♂ and 14.0 mm. for ♀. In the Chinese skulls measured by Sir Arthur Keith it is 12.5 mm., and in Naga Group I it is 13.5 mm.

The difference between the projection of the nasion and

TABLE XLVI.—BURMESE CRANIA (FEMALE).
Facial Measurements in an Antero-posterior Plane.

Skull No.	A	B	C	D	E	F	G	H	I	J
422	84	86	84	80	85	77	84	88	66	16
424	87	92	70	85	90	83	87	92	70	14
426	83	88	66	81	85	79	85	90	62	13
435	86	88	66	83	83	77	80	83	64	14
436	88	93	69	86	90	82	87	87	68	18
465	85	82	66	82	82	77	82	85	61	—
Average	85.5	88.0	70.0	82.8	85.8	79.0	84.0	87.5	65.0	15.0

Explanation of heading letter of each column :

- A. Projection of the glabella in front of the mid-meatal plane.
- B. Extent to which the tip of the nose lies in front of the mid-meatal plane.
- C. Projection of the lateral orbital margin in front of the mid-meatal plane.
- D. Projection of the nasion in front of the mid-meatal plane.
- E. Projection of the most advanced point in the ascending nasal process of the maxilla in front of the mid-meatal plane.
- F. Projection of the least advanced part of the lateral margin of the pyriform aperture in front of the mid-meatal plane.
- G. Projection of the least advanced point just below the nasal spine in front of the mid-meatal plane.
- H. Projection of the upper alveolar point in front of the mid-meatal plane.
- I. Projection of the lower malomaxillary point in front of the mid-meatal plane.
- J. Height of the most advanced part on the dorsum of the nose from the inferior orbital margin.

the projection of the upper alveolar point from the mid-meatal plane gives us a very useful method for determining the nature of alveolar prognathism. This upper alveolar prognathism is 4.9 mm. for ♂ and 4.7 for ♀ in the present series. In Naga Group I it is 8.2 and in the Chinese 4.5, showing that our series is less prognathic than Naga Group I,

but almost equal to that of the Chinese measured by Sir Arthur Keith.

Compared, therefore, with the Chinese skulls studied by Sir Arthur Keith and the Naga skulls investigated by us,

TABLE XLVII.—BURMESE CRANIA (FEMALE).
Projection of the Cheek-bones.

Skull No.	A	B	C	D	E	F
422	72	66	76	66	38	18
424	79	73	83	72	45	21
426	75	69	77	63	45	23
435	74	68	78	66	44	24
436	76	71	78	69	40	18
465	72	70	73	62	43	22
Average	74.2	69.5	77.5	66.3	42.5	21.0

Explanation of heading letter of each column :

- A. The radial distance of the anterior end of the frontomalar suture from the transmeatal axis.
- B. The radial distance of the middle point on the lateral margin of the orbit from the transmeatal axis.
- C. The radial distance of the point on the lower margin of the orbit crossed by the malomaxillary suture from the transmeatal axis.
- D. The radial distance of the malomaxillary point from the transmeatal axis.
- E. The distance of the lower malomaxillary point from the anterior frontomalar point.
- F. The distance between the lower malomaxillary point and the nearest point on the lower margin of the orbit.

we find that in all the three groups there is a remarkable retrocession of the lateral nasal walls, together with a forward projection of the zygomatic bones, giving the face the characteristic Mongoloid flatness. Below are given the composite profile views of the Burmese ♂ and ♀ (figs. 156, 157). The ♂ profile view is based on 30 well-preserved skulls and that of ♀ on 6 skulls. It will be seen from these drawings that both the ♂ and ♀ skulls show the typical

Mongolian flatness of the face as described above and are in this respect comparable to the profile view of the Naga

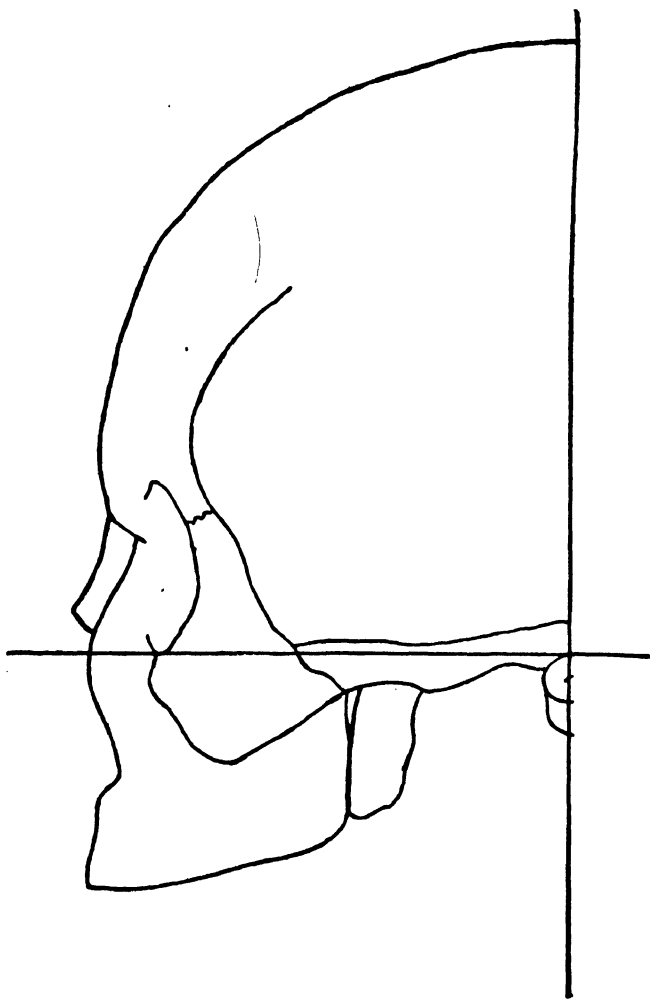


FIG. 156. Composite facial profile of the Burmese males, $\times \frac{1}{3}$.

Group I, though the latter are dolichocranial and not brachycranial like the present series.

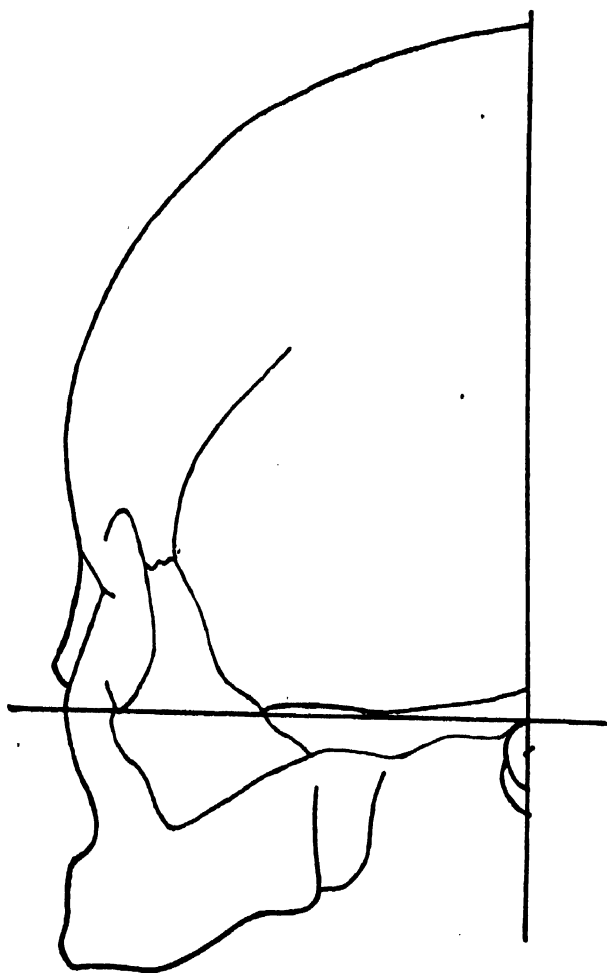


FIG. 157. Composite facial profile of the Burmese females, $\times \frac{1}{2}$.

TABLE XLVIII.—BURMESE CRANIA (MALES).

Facial Measurements in an Antero-posterior Plane.

(For explanation of letters, see Table XLVI.)

Skull No.	A	B	C	D	E	F	G	H	I	J
421	85	88	67	83	86	77	82	84	65	15
423	94	93	71	90	91	82	88	88	67	17
425	90	98	71	87	91	81	86	88	64	19
427	88	87	68	83	85	80	87	90	66	15
428	96	98	71	93	94	85	90	92	69	20
429	87	94	67	86	92	85	91	96	66	21
430	89	91	69	84	89	82	88	96	65	17
431	84	89	70	81	89	83	87	90	68	15
432	89	96	74	85	93	85	91	97	68	17
433	96	94	75	90	91	85	89	87	74	18
434	91	98	70	90	93	88	93	101	72	16
437	83	88	67	83	85	81	86	90	66	15
450	93	98	71	90	93	86	91	94	69	18
451	85	86	67	82	88	80	86	90	64	11
452	97	97	74	93	95	89	92	93	72	15
453	79	86	62	78	86	79	82	81	60	18
454	83	94	65	82	88	82	88	89	63	21
455	80	81	64	79	81	72	78	76	60	12
456	82	86	67	79	85	82	87	91	65	11
457	91	95	68	87	90	82	86	88	62	19
459	87	91	63	82	87	80	83	87	63	21
460	93	101	76	91	98	89	96	101	77	18
461	92	91	70	84	89	79	86	87	62	18
462	88	97	70	87	93	84	92	92	74	18
463	91	96	73	90	95	90	91	98	70	15
464	91	98	72	89	92	84	90	91	64	17
466	89	96	71	87	92	86	93	96	70	17
467	85	83	68	83	84	81	86	90	65	13
468	82	94	68	82	89	82	86	87	66	18
495	96	96	72	95	92	81	90	90	69	19
Average	88.5	92.7	69.4	85.8	89.9	82.7	88.0	90.7	66.8	16.8

TABLE XLIX.—BURMESE CRANIA (MALES).

Projection of the Cheek-bones.

(For explanation of letters, see Table XLVII.)

Skull No.	A	B	C	D	E	F
42I	73	69	75	67	44	22
423	8I	75	8I	68	45	22
425	77	73	8I	64	44	27
427	77	70	75	67	4I	20
428	8I	74	82	70	44	23
429	78	7I	79	68	49	24
430	79	72	78	67	45	2I
43I	79	73	77	69	36	20
432	83	76	82	7I	49	26
433	82	77	80	77	48	25
434	80	76	84	73	49	26
437	73	69	76	67	42	2I
450	79	73	79	70	4I	20
45I	78	7I	78	66	44	22
452	82	75	86	73	42	22
453	73	64	7I	63	48	24
454	76	69	75	65	46	23
455	73	68	72	63	44	22
456	78	72	78	65	44	2I
457	76	7I	78	64	46	28
459	74	67	72	64	43	20
460	84	78	85	80	48	24
46I	79	7I	75	63	44	2I
462	77	72	80	75	43	I9
463	80	75	83	73	45	27
464	80	76	8I	66	45	27
466	79	73	8I	7I	4I	22
467	80	73	77	66	46	23
468	79	70	76	68	47	24
495	84	75	80	70	43	I9
Average .	78.5	72.3	78.6	68.4	44.5	22.8

CONCLUSION

From the foregoing accounts it is found that the Burmese skulls, obtained from an old burial-ground in Prome described above, are definitely Mongoloid, and the dominant type is broad-headed and broad-nosed, although a few long-headed skulls are also present as in No. 427, C.I. 73·4; No. 450, C.I. 74·2; No. 460, C.I. 72·7; and No. 495, C.I. 71·7. Compared with the Burmese Proper measured by Turner and Tildesley, our series agrees very closely in regard to the maximum length and breadth of the skulls, though they are somewhat lower vaulted. The nose form does not differ very much from those of Tildesley, but the nasal index is significantly higher than that of Turner. The orbits of the two sides are variable, but they are usually medium in size. The average orbital index is higher than the Burmese Proper of Tildesley, but lower than that of Turner. The zygomatics are prominent and the face is characteristically flat. The average maximum bizygomatic breadth is slightly shorter than in the groups of both Turner and Tildesley. The crowns of the teeth are often slightly eroded and in only one skull are there signs of caries. The palates are usually broad and are decidedly broader than the Burmese Proper of Tildesley. The face, generally speaking, is either Mesognathous or Orthognathous; only four of them are Prognathous, though they are comparatively more prognathic than the group of Tildesley. In their facial projection they show a remarkable retrocession of the lateral nasal walls, together with a forward projection of the zygomatic bones, giving the face a characteristic Mongoloid flatness.

In explaining the lack of physical homogeneity among the Burmese, Sir William Turner¹ suggested 'a certain admixture with the brachycephalic Burmese of a race or races with dolichocephalic proportions of the skull is to be found in Burma. It is possible that they may be descendants of the aboriginal people, or be those of persons, or the descendants of persons, who had migrated into Burma from

¹ Sir William Turner, *loc. cit.*, p. 736.

the hill districts at present inhabited by a dolichocephalic race ' like the Nagas described by us. This is also the view borne out by the present study, and there does not appear any reason to doubt that the prevailing Burmese type is brachycephalic Mongolian, with a certain amount of admixture with a dolichocephalic type probably derived, as suggested by Turner, from the neighbouring hill tribes.

In carrying on this work I am deeply grateful to Sir J. C. Bose, F.R.S., Director of the Bose Research Institute, for his sympathetic encouragement and advice. I am also obliged to the Director of the Z.S.I. for permission to work on these skulls, and to Dr. B. S. Guha, Anthropologist to the Z.S.I., under whose directions the work was carried out ; and, lastly, for the kind permission of the Director of the Zoological Survey of India to publish the exact reproductions of the skulls photographed by Professor N. C. Nag and Mr. N. N. Das.

XVII.—INVESTIGATIONS ON THE RADIO-ACTIVITY OF HOT SPRINGS AT RAJGIR

BY

N. C. NAG, M.A., F.I.C.

RAJGIR is in the district of Patna in the Indian Province of Bihar and Orissa. It is surrounded by five hills—*Bipulachal*, *Ratnagiri*, *Udayagiri*, *Sonagiri*, and *Baibhar*. These hills are mentioned in the ancient epic Mahabharata under the names of *Baihar*, *Baraha*, *Brishava*, *Rishigiri*, and *Chaityaka*. At the foot of some of these hills hot springs rush out from subterranean depths. The waters of the springs have, from very ancient times, been regarded as very sacred and credited with curative properties for various human ills. Even at the present time the springs at Rajgir attract numerous pilgrims. The waters of the different spouts and pools are held to have specific curative properties and are supposed to have different origins. Amongst the most important of these are the *Saptadhara* springs and the adjoining pool of *Brahma-kund*.

Sir J. C. Bose visited Rajgir more than a quarter of a century ago, and carried out certain investigations on the *Saptadhara* springs, as well as on the *Brahma-kund* pool. It has been supposed that these had different origins, apparently supported by the fact that the temperatures of the waters were different. But careful examination led him to conclude that this was not so, the temperature being the same in all cases. The apparent variation he ascribed to the different paths and distances the water had to travel before finding outlets through the spouts. This led him to suppose that the *Saptadhara* springs and the

Brahma-kund pool must arise from the same source situated deep down in the rocks, the activity of which is practically inexhaustible. Facts will presently be given which strongly support this view.

In regard to the supposed curative effect of the spring water, could it be a mere instance of faith cure, or might it be due to some active substance present in the water ?

The detection of active emanations has been rendered possible by means of relatively new methods of investigation, and, at Sir J. C. Bose's request, I undertook the work of examining the water of the hot springs at Rajgir for detection of active substances, to which might be due the reputed curative property so often associated with water of hot springs in other places.¹

Two scientific expeditions, under Sir J. C. Bose, left for Rajgir, the first in January 1930, and the second in January 1931; the results of the work entrusted to me are described below. It may be added that observations of January 1930 were fully confirmed by those of 1931, which had the advantage of previous experience and knowledge of the conditions prevailing at the place.

His Excellency Sir Hugh Stephenson, Governor of Bihar and Orissa, has been very kind in offering special facilities for carrying out the investigations.

PRESENCE OF RADIO-ACTIVE MINERALS

The question arises : How could the spring water contain active emanation unless radio-active minerals were present in the rocky beds through which the springs are flowing ? In regard to this it may be urged that at one time the Singar Mines gave promise of being an important source of Uranium, and hence of Radium. The Singar Mines are in the neighbourhood of Rajgir, the latitude and longitude of the former being $25^{\circ} 1'$ and $85^{\circ} 29'$, while those of the latter are $24^{\circ} 34'$ and $85^{\circ} 33'$ respectively.² A few years ago I had the

¹ Stefan Meyer and Egon Schweidler, *Radioaktivität*, pp. 367 *et seq.*

² La Touche, 'Annotated Index of Minerals of Economic Value,' *Geological Survey of India*.

opportunity of analysing some radio-active columbite from neighbouring localities,¹ and very recently I have been able to detect radio-active properties in some rare-earth minerals from Ranchi, which is not far from Rajgir.

THE TEMPERATURE OF THE WATER OF THE SPRINGS

There are two important springs, of which the *Saptadhara* and the adjoining *Brahma-kund* pool are under Hindu management. The second spring, *Makhdum-kund*, at a distance of about a quarter of a mile from *Saptadhara*, rushes out from the foot of another hill, and is under Mohammedan management.

I carefully examined the temperature of the water of the different springs, the temperature of the surrounding air being about 56° F. The following give the results of observations :

<i>Saptadhara</i> spout temperature	.	.	108° F.
<i>Brahma-kund</i> pool temperature	.	.	108° F.

The temperatures of the distant spring *Makhdum-kund* are :

<i>Makhdum-kund</i> spout temperature	.	.	98° F.
<i>Makhdum-kund</i> pool temperature	.	.	97° F.

It is a matter of very great scientific interest to find that the temperature of the water of the springs has remained practically unchanged for at least 100 years. This appears from the observations of Dr. Francis Buchanan, who describes the results obtained by him more than 100 years ago. It is to be borne in mind that the thermometer employed by Buchanan was necessarily different from ours, and that on this account there might be some slight difference between the readings of the two.

In regard to his readings, taken in degrees F., he says :

' In *Brahma-kund* the water is collected in a pool. . . . On the 19th of January, the thermometer in the air being

¹ Nag, 'Radio-Active Columbite from Gaya District,' *Jour. Geolog. Min. and Metallurgical Soc. India*, 1929, vol. ii, pt. i.

62°, it rose in the water to 109°. The water from all the spouts is perfectly clear. In that called *Kashi* the thermometer stood at 107° F.'

The result, 108° F., obtained by us for the pool and the spout, is thus practically the same as that obtained by Buchanan 100 years ago.

In regard to the temperature of the water of the *Sringgi Rishi-kund*, now known as *Makhdum-kund*, appropriated by the Mohammedans, Buchanan¹ says :

'The remaining pool, *Sringgi Rishi*, is situated at the foot of the hill, about a quarter of a mile east of the others, and its heat is only 97° F.'

My observation of the temperature of *Makhdum-kund* is also 97° F.

From these results it would appear that—

- (i.) The source of the spring at *Saptadhara* and of the pool at *Brahma-kund* is the same, and that the out-flow of energy from this source is inexhaustible, since the temperature had remained constant for such a very long period.
- (ii.) The source of the spring at *Makhdum-kund* may be different, as it rushes out at the foot of another hill ; as a matter of fact the temperature of its water is not the same as that of *Saptadhara* and of *Brahma-kund*.

CHEMICAL ANALYSIS OF WATER

The water examined was found to be exceptionally pure² and free from organic substances. Examination of water, after being kept for 17 months, showed no presence of micro-organisms.

Examinations of the *Saptadhara* water showed :

Total solids . . . 3 parts in 100,000.

¹ Quoted in *History, Topography and Antiquities of Eastern India*, by Montgomery Martin (London, Feb. 1838), vol. i, p. 256.

² Thermal spring waters with very pronounced physiological effects, which are said to be approximately proportional to the amount of emanation in the water, do not show any particular chemical composition. (Bad, Gastein, Plombière. Cf. Madame Curie, *Treatise on Radio-Activity* (1910), vol. ii, p. 206 ; also Rutherford, *Radio-Activity* (Berlin, 1907), p. 225.)

It was practically all silica, with nothing else in any appreciable quantity.

Brahma-kund pool gave on the same day :

Total solids 8 parts in 100,000.

Saptadhara water (3 litres) collected on January 21 gave :

Total solids 5·8 parts in 100,000.

A quantity of 2890 c.c. *Saptadhara* water was kept in a clean, stoppered, hard glass bottle just as it was collected on January 21, 1930. The water was perfectly clear, with no sediment. When examined on June 18, 1931—that is to say, after about 17 months—the water was still clear, but a gelatinous film had settled down at the bottom of the bottle. This was taken, after filtration, from the main body of water and weighed in a platinum basin, after drying. After ignition it showed no change and was found to be silica. Evidently this portion of the solid matter was in a dispersed state of colloidal solution. The result came out as 0·18 per 100,000 parts.

A portion of the filtrate was then evaporated for solids and gave 5·60 in 100,000 parts. Therefore total solids for *Saptadhara* water after 17 months works out as follows :

Total solids (*Saptadhara*, after
17 months) 5·78 per 100,000.

The following is the result of analysis made by Dr. K. N. Bagchi, the Chemical Examiner of Patna :

	Brahma-kund	Saptadhara	Makhdum-kund
Total Solids	8·0	5·8	7·2
Chlorine	0·9	0·8	0·9
Free Ammonia N	Minute	Nil	Nil
Albuminoid N	Nil	Nil	Nil
Nitrate and Nitrite N	0·01	0·01	0·02
Other substances, such as phosphate	—	—	Nil
Iron and sulphate in merest traces			

SOURCES OF SPRING WATER AND GAS

The following are the different springs and pools from which water and evolved gas were collected for examination. The streams which flow out of *Saptadhara* and the pool *Brahma-kund* are, as already stated, in close contiguity. The floor of the pool *Brahma-kund* is covered with layers of pebble; gas from some subterranean source bubbles out through the pebbles in an intermittent manner. The gas thus evolved was carefully collected for examination. The water flowing out of the *Saptadhara* was similarly collected. Water of the spring *Makhdum-kund* was also collected for examination.

METHOD OF INVESTIGATION

It is necessary to employ a modern method of investigation for the discovery of the possible presence of some active emanation in the water of the hot springs. "This depends on the fact that radium produces a gaseous product, the emanation, of a comparatively long period of transformation which can be separated completely from radium solutions. The emanation reaches the equilibrium value after the radium solution has been sealed up in an enclosure for about a month. When this stage is reached the amount of emanation is proportional to the radium content of the solution." The presence of emanation is found from the change in the rate of natural leakage of electric charge of an emanation electroscope after the introduction of the emanation from the spring water or of the evolved gas into the ionisation chamber, which "increases the rate of leak, at first rapidly and then more slowly, reaching a maximum. . . . During this interval it is advisable to keep the leaf of the electroscope negatively charged so as to concentrate the active deposit on the central rod. . . . For accurate work it is desirable to take measurements of the leak when at its maximum."¹ If the maximum rate of leak is reached in about 3 hours, then the emanation is of radium origin.

¹ Glazebrook, 'The Radium Emanation Method,' *Dictionary of Applied Physics*, vol. iv, p. 631 et seq.

"The emanation method is very accurate and reliable. The emanation from 10^{-6} mgr. of radium gives a comparatively rapid discharge and it is possible to measure one-hundredth of this quantity with certainty."¹

The instruments employed and their mutual relation are diagrammatically represented in fig. 158. The gas or the water for examination is contained in the flask F, U being an interposed drying tube.

For holding the standard radio-active emanation, the special wash bottle A, with the outer jacket, is employed.

A more detailed account of the electroscope, and the method of charging, as well as the method of observation, is given below.

THE GOLD-LEAF ELECTROSCOPE

A suitable portable Gold-Leaf Electroscope of high sensitivity was constructed at the Bose Institute Workshop ;

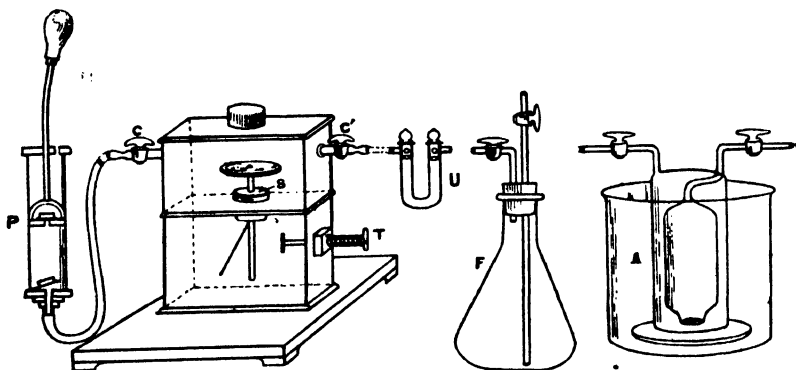


FIG. 158.—Electroscope in working order.

P, hand pump ; C, C', stopcocks ; S, insulating sulphur rod ; T, charging tapper ; U, drying tube ; F, flask for collecting gas and water to be examined ; A, specially constructed glass flask for standard radio-active emanation.

The positions of F and A are interchangeable ; the one is connected with the drying tube while the other stands by.

a diagrammatic representation is seen to the left of fig. 158. The electroscope is two-chambered, the upper one being

¹ Rutherford, *Radiations from Radio-active Substances* (1930), p. 565, refers to Rutherford and Boltwood Method, *Amer. Jour. Sci.*, 1905, vol. xx, p. 55 ; vol. xxii, p. 1, 1906.

provided with two stopcocks C, C', by proper manipulation of which it can be evacuated by working the hand pump P,¹ or filled with gas or emanation after passing through the drying tube as required. S is the rod of sulphur through which passes the metallic strip with the gold leaf. The inter-space between the metal and the sulphur is rendered airtight by means of thin rubber bands. The lower chamber of the electroscope has a glass window, which can be opened and closed at pleasure and is utilised for ordinary α -ray examination. Opposite to this glass window is fixed a glass strip which allows light, coming from an ordinary electric torch light placed at a distance of about 30 cms., to enter the electroscope and thus make visible the gold leaf and the scale divisions. Deflection of the leaf is observed in the usual manner by a reading microscope provided with a micrometer scale suitably placed in the eye-piece.

The gold leaf is charged by pushing the tapper T, suitably insulated in the lower chamber; this tapper is connected with the negative end of the charging battery, the positive end being connected with the outer metallic case which is kept earthed. The tapper, after the process of charging, is disconnected from the negative and allowed to fly back to its normal position in connection with the outer casing, thus becoming earthed. A dry battery as usually employed in wireless receiving apparatus for high tension is quite effective for the purpose of charging. A maximum of 120 volts suffices for all ordinary purposes. For comparative readings, the time of fall between two definite scale readings is noted every time; other usual precautions, such as allowing full time for creeping and attainment of constant stage, are also taken.²

With the particular electroscope that was used in the 1930 experiments at Rajgir, change of deflection of the gold

¹ Instead of a hand pump it is quite convenient to use a rubber circulating pump as described by Meyer and Schweidler; it is then not necessary to suck out the air in the emanation chamber, nor should the chamber be very large in capacity. The same volume of air passing and repassing several times over carries all the emanation to the charged electroscope.

² Glazebrook, *ibid.*; Makower and Geiger, *Practical Measurements in Radio-Activity*.

leaf, due to natural leakage, was fairly regular between scale divisions 46 to 22, as the voltage fell from 120 to 80. Observations were, however, continued to scale reading 8.5. The voltage and deflection curve is given in fig. 159.

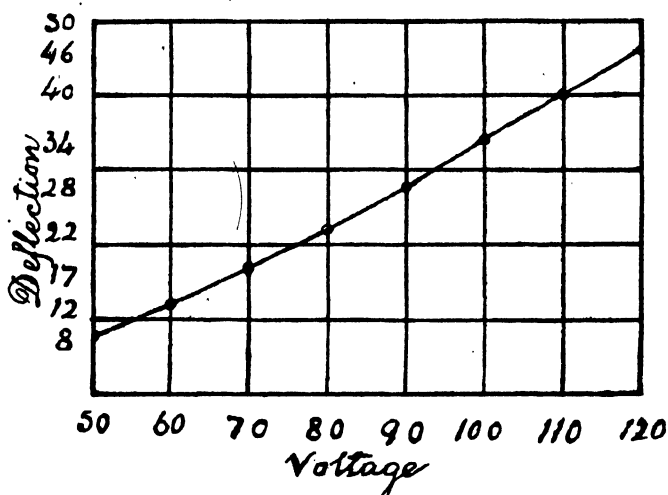


FIG. 159. Voltage and deflection curve of the charged gold leaf.

The natural leak is ascertained from observation of the fall of deflection of the charged gold leaf at definite intervals of time, say one hour. If the introduction of air, bubbling through the spring water, into the ionisation chamber of the electroscope brings about an increase in the rate of leak, then the water must contain radio-active emanation.

OBSERVATION FOR DETERMINING THE RATE OF NATURAL LEAK

This was found by observation of the change of deflection of the particular gold leaf at different intervals, as given in the following tabular statement. The last result, within brackets, was obtained by extrapolation.

Time in hours from start	Deflection readings
0	46
4	41
8	37
12	33.5
16	30
20	27
24	23.5
32	19
40	15
(52)	(10)

The *average natural leakage* is only 0.69 division per hour when calculated over the whole range of the scale from 46 to 10 (fig. 160). It must be noted that *natural leak*, expressed

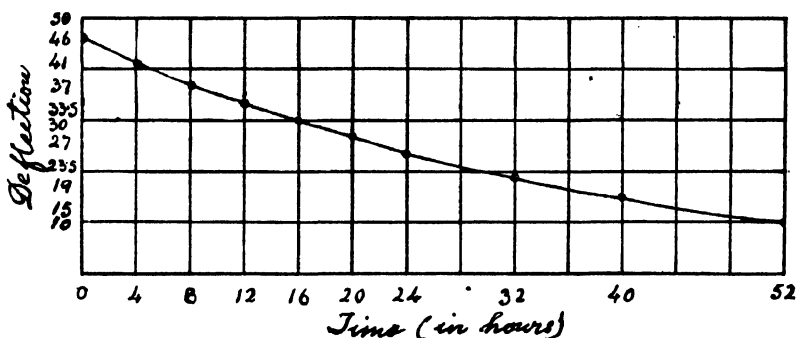


FIG. 160. Natural leak curve.

in divisions per hour, will vary according to the scale divisions (or voltage ends) between which the times were noted. In the present case natural leak works out practically to one division per hour between scale divisions 46 and 23.

The portable apparatus was taken to Rajgir. As the results already obtained with the particular electroscope exhibited a very low rate of leakage, and since the observations taken over a wide range necessitated prolonged time of observation, it was thought advisable to shorten the period of experiment by observing the fall of deflection over

only two scale divisions at the high-voltage end. The following data were obtained at Rajgir :

January 13, 1930.

(1) Time of observation	Deflection
5.20 A.M.	46
7.0 A.M.	44

That is to say the natural leak was 2 divisions in 100 minutes or 1.20 divisions per hour.

A second set of readings taken after recharging at noon gave the same results :

(2) Time of observation	Deflection
12.20 P.M.	46
2.0 P.M.	44

The rate of *natural leak* was thus found to be constant, being 1.20 divisions per hour.

METHOD AND ORDER OF INVESTIGATIONS

After the determination of the rate of leakage, two different lines of investigation were employed for the detection of radio-active emanation present in the water, and the character of the radio-active substance, by :

- (a) the induced enhancement of the rate of natural leak, as well as
- (b) the increasing rate of leakage culminating in a maximum after a definite time, about three hours, indicating radium emanation.

The question arises : how is the emanation present in the spring water to be introduced into the ionisation chamber ? This is done by drawing in air-bubbles through the specimen of water. But moist air, being more conducting, causes a more rapid discharge and increased leakage. This defect is obviated, as will be explained presently, by passing the air through drying tubes. The reliability of the method is found from the following tests :

- (1) The air bubbling through ordinary water is introduced, after drying, into the ionisation chamber. This is found to induce no enhancement in the normal rate of leakage.
- (2) The air bubbling through spring water is next introduced, after drying, into the ionisation chamber, with the result that the rate of normal leakage becomes enhanced. This is a proof of the presence of radio-active emanation in the spring water.

After explaining the principle of the methods employed I give below a list of investigations which were carried out in the following order :

Experiments of 1930.

- (1) Detection of radio-activity of *Brahma-kund* gas from the induced enhancement of the rate of leakage.
- (2) Examination of *Brahma-kund* water by the method of leakage.
- (3) Determination of the nature of the radio-active *Brahma-kund* gas.
- (4) Radio-activity units and standards.

Experiments of 1931.

- (5) Determination of the natural leakage of the new electroscope.
- (6) Radium-emanation standard for 1931.
- (7) Examination of *Brahma-kund* gas.
- (8) Relative activities of the different spring waters.
- (9) Activity expressed in emanation units.

I. DETECTION OF RADIO-ACTIVITY OF BRAHMA-KUND GAS FROM THE INDUCED ENHANCEMENT OF RATE OF LEAKAGE

About 20 c.c. of gas was collected from the *Brahma-kund*; the gas was, after drying in the usual manner (*see below*), introduced into the already electrically charged evacuated ionisation chamber of the electroscope at 6.30 P.M. It is to

be remembered that the gas introduced was not changed, the observation consisting in determining whether the rate of leakage underwent a progressive increase for a time from the beginning.

January 13, 1930.

(1) Time of observation	Deflection
6.35 P.M.	46
7.15 P.M.	40

The fall of the gold leaf was thus 6 divisions in the course of 40 minutes. This gives the rate of leakage as 9 divisions per hour.

The electroscope was immediately recharged to full voltage, and observation made once more, starting from the deflection of 46.

(2) Time of observation	Deflection
7.16 P.M.	46
7.52 P.M.	40

The rate of leakage had thus increased further to 6 divisions in 36 minutes or to 10 divisions per hour. The natural rate of leakage shown by the electroscope was, as previously determined, 1.2 divisions per hour. The introduction of the gas enhanced the rate of leakage to 9 or even 10 divisions per hour, offering conclusive proof of radio-active emanation present in the gas.

After each experiment the ionisation chamber has to be evacuated and dry air introduced into it, the electroscope being thus allowed to recover its natural rate of leakage. It is only after this that the next observation is undertaken.

2. EXAMINATION OF BRAHMA-KUND WATER BY THE METHOD OF LEAKAGE

Immediately after the determination of the rate of natural leakage, which was 1.20 divisions per hour, 60 c.c. of *Brahma-kund* water was collected in a flask F; air was then slowly bubbled through it into the previously evacuated

ionisation chamber, being dried during its passage through drying tubes U, containing soda-lime and calcium chloride. It may be stated that the capacity of the ionisation chamber is much larger than the volume of the water flask in which the water was collected, and through which the air was bubbled. After the ordinary atmospheric pressure was restored, the stopcocks C, C' were closed, and the pump P and other connections were disconnected. It should be noted that during the process of introducing air through the water under examination into the ionisation chamber, the gold leaf was kept charged negatively, while the outer casing was kept earthed and connected to the positive end.

January 15, 1930.

Time of observation				Deflection
3.30 P.M.	.	.	.	46
4.52 P.M.	.	.	.	44

The rate of natural leakage has in a previous experiment been found to be 1.2 divisions per hour, during the fall of the gold leaf from 46 to 44 divisions. After the introduction of air bubbling through the *Brahma-kund* pool water, the same fall occurred in 92 minutes, or at the rate of 1.3 divisions per hour. This result points to a slight increase in the rate of fall of the gold leaf, on account of the presence of radio-active emanation in the water, the activity of which is not so pronounced as that of the evolved gas.

3. DETERMINATION OF THE NATURE OF THE RADIO-ACTIVE BRAHMA-KUND GAS

A stop watch reading to one-fifth of a second was used in noting the time of fall of the gold leaf from 46 to 10 divisions in every successive observation. The volume of dried gas introduced into the ionisation chamber was 60 c.c. The usual method of charging and recharging was followed for each observation.

TABLE L.—JANUARY 18, 1930.

Time of observation	Minutes	Calculated activity per hour
(1) 2.30 P.M. to 2.51.5 P.M.	21.5	99.78
(2) 2.53 " " 3.11 "	18	119.31
(3) 4.13 " " 4.29.6 "	16.6	129.43
(4) 5.8 " " 5.24 "	16	134.31
(5) 5.25 " " 5.40.8 "	15.8	136.02
(6) 6.14 " " 6.32 "	18	119.31
(7) 8.50 " " 9.9 "	19	112.99

From the above it will be noted that the activity increased at first rapidly and then more slowly, reaching the *maximum value in about three hours*. Similar results were obtained on repeating the experiment on January 20, 1930.

The fact that the maximum radio-activity was reached in about three hours points to the emanation being of radium origin.

4. RADIO-ACTIVITY UNITS AND STANDARDS

For a comparative study, a weighed quantity of standard Joachimsthal pitchblende, kindly supplied by Dr. D. M. Bose, containing 0.617 grm. of uranium per gram of the mineral and therefore 2.1×10^{-4} mgr. of radium, was taken and dissolved in nitric acid and hydrochloric acid, evaporated to dryness, and finally the residue redissolved in dilute hydrochloric acid. The solution was so diluted as to contain 1.05×10^{-5} mgr. of radium in 50 c.c.; this was carefully kept in a stoppered glass bottle (fig. 158, A), to ensure against leakage, the bottle being interchangeable with the flask F, mentioned before, and capable of easy manipulation for pumping the emanation into the upper chamber of the electroscope whenever this is required.¹ In the present case the solution was kept sealed up from February 7, 1930,

¹ Makower and Geiger, *ibid.*, pp. 111, 114.

to March 9, 1930, when the activity curve of the emanation from the standard solution was worked out.

It may be noted that *the quantity of emanation in equilibrium with one gram of radium is known as a Curie of emanation.*

Again, 10^{-18} Curie of emanation per c.c. is known as one Eman.¹

Effect of emanation from the standard solution.—The emanation from this solution was introduced into the ionisation chamber with the usual precautions on March 9, 1930, after determination of the rate of natural leakage, and the following observations recorded for the fall of the gold leaf from 46 to 10 divisions.

TABLE LI.—MARCH 9, 1930.

Time of observation				Minutes	Calculated activity per hour
(1)	8.30	A.M. to	8.45 A.M.	15	143.31
(2)	9.1.4	" "	9.14.2 "	12.8	168.06
(3)	9.15.6	" "	9.28 "	12.4	173.52
(4)	10.19.4	" "	10.31 "	11.6	185.51
(5)	10.32.4	" "	10.43.8 "	11.4	188.79
(6)	10.44.8	" "	10.56 "	11.2	191.17
(7)	11.19	" "	11.30.2 "	11.2	191.17
(8)	11.44.8	" "	11.56.4 "	11.6	185.51
(9)	11.57.6	" "	12.10 P.M.	12.4	173.52

The results given above of the variations of activity of the emanation from the standard solution may be compared with those obtained with 60 c.c. of *Brahma-kund* gas on January 18, 1930, which has already been given on page 333. Fig. 161 gives the results plotted in two comparable curves, the upper being that due to the standard emanation and the lower that due to *Brahma-kund* gas. The nature of the two curves is so similar, with their respective maxima

¹ St. Meyer and E. Schweidler, *ibid.*, p. 565.

occurring about three hours after commencement, that it leaves no doubt as to the radio-activity being due to radium emanation.

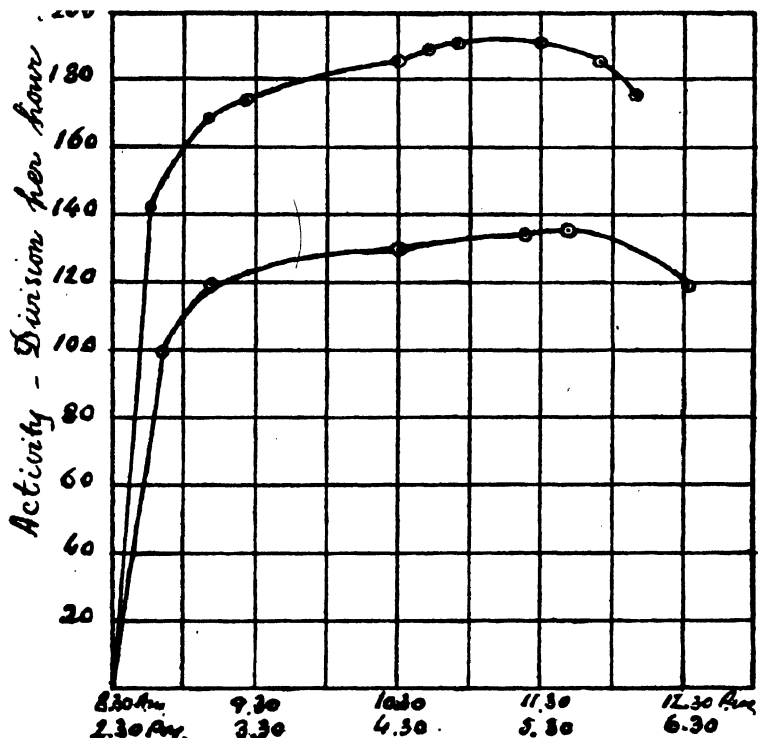


FIG. 161. Upper curve : Activity of standard solution.
Lower curve : Activity of Rajgir *Brahma-kund* gas.
Both exhibit maximum in about 3 hours.

The relative activity figures for *Brahma-kund* gas and the standard solutions are 136.02 and 191.17.

5. DETERMINATION OF THE RATE OF NATURAL LEAKAGE OF THE NEW ELECTROSCOPE

The experiments that follow were carried out in 1931, the Gold-Leaf Electroscope employed being of the same design as that of 1930 ; its ionisation chamber had, however,

a much larger volume capacity. The fall of the gold leaf was noted between 46 and 23 divisions. In order to adjust the deflection to the scale division 46, it was only necessary to charge the electroscope from a 108-volt dry battery.

Observations of the rate of natural leakage obtained with the new electroscope gave the following results :

January 4, 1931.

Time of observation	Deflection
6.15 A.M.	46
8.42 A.M.	35
11.38 A.M.	23

The experiment was repeated on the following day.

January 5, 1931.

Time of observation	Deflection
6.35 A.M.	46
8.35 A.M.	37
12.0 noon	23

It will be seen that the average time required for fall of deflection from 46 to 23 divisions on account of natural leak is 324 minutes. The rate of natural leakage thus works out as 4.2 divisions per hour.

6. RADIUM EMANATION STANDARD FOR 1931

The standard used was the same as in the previous year. The same procedure was followed as in the previous year for introducing the emanation into the ionisation chamber with the usual precautions of evacuating, charging the electroscope, drying the emanation before entrance into the chamber, as was done in every such case (*see* pages 331-334).

Effect of emanation from the standard solution.—After following the usual procedure, readings of time were taken for the fall of deflection from 46 to 23, the electroscope being recharged every time to get the full deflection 46 before commencement of the next time-observation. The following readings were taken :

TABLE LII.—JANUARY 6, 1931.

Time of observation					Minutes	Calculated activity per hour
(1)	8.30	A.M. to	8.35	A.M.	5	271·8
(2)	8.41·6	„ „	8.45·6	„	4	340·8
(3)	8.56	„ „	8.59·8	„	3·8	359
(4)	9.16	„ „	9.19·5	„	3·5	390·1
(5)	9.43	„ „	9.46·3	„	3·3	414
(6)	10.12	„ „	10.15·1	„	3·1	440·9
(7)	10.28	„ „	10.31	„	3	455·8
(8)	10.58	„ „	11.0·9	„	2·9	471·7
(9)	11.12	„ „	11.14·8	„	2·8	488·6
(10)	11.16	„ „	11.18·8	„	2·8	488·6
(11)	11.25	„ „	11.28	„	3	455·8
(12)	11.31	„ „	11.34·1	„	3·1	440·9

The maximum activity of emanation from the standard solution was reached at or very near 11.19 A.M., *i.e.*, 2 hours and 49 minutes after introduction of the radium emanation into the ionisation chamber.

For observation once more of the rate of natural leakage, the chamber was quickly evacuated and filled with dry air. On January 7, 1931, the fall of the leaf from 46 to 23 was effected in 325 minutes, between 7.35 A.M. and 1 P.M., which is practically the same as the result obtained previously (*cf.* p. 336). This allowance for rate of natural leakage has to be made for calculating the radio-activity of the different gases.

7. EXAMINATION OF BRAHMA-KUND GAS

Observations were next made with the gas evolved from *Brahma-kund*. In this instance the capacity of the flask F. was 350 c.c. Fifty c.c. of gas were collected with 300 c.c. of water from the *Brahma-kund* pool. Proceeding in the same manner as with the standard radium emanation, the 50 c.c. of gas, with whatever emanation there was in solution in the 300 c.c. of water, were introduced into the evacuated

ionisation chamber of the charged electroscope, the recorded observations being as follows :

TABLE LIII.—JANUARY 8, 1931.

Time of observation				Minutes	Calculated activity per hour
(1)	8.5	A.M. to	8.12 A.M.	7	192.9
(2)	8.30	„ „	8.35.5 „	5.5	246.7
(3)	9.2	„ „	9.7 „	5	271.8
(4)	10.1	„ „	10.5.8 „	4.8	283.4
(5)	10.38	„ „	10.42.7 „	4.7	289.4
(6)	10.44.2	„ „	10.48.7 „	4.5	302.4
(7)	10.50.2	„ „	10.54.7 „	4.5	302.4
(8)	10.56.3	„ „	11.1 „	4.7	289.4

The maximum activity was attained in about 2 hours 50 minutes, the gas having been introduced at 8.0 A.M. This result is practically the same as that obtained in 1930, already given on p. 333.

8. RELATIVE ACTIVITIES OF THE DIFFERENT SPRING WATERS

It has been stated that the spring *Makhdum-kund*, appropriated by the Mohammedans, and situated at some distance from the *Brahma-kund*, has probably a different origin, since its temperature is about 98° F., in place of 108° F. of *Brahma-kund*. The investigations carried out in 1931 were for the approximate determination of relative radio-activity of the waters in *Brahma-kund*, *Makhdum-kund*, and *Saptadhara*. Observations were made on January 13, 14, and 16, 1931, respectively, the method employed being the determination of maximum activity. For the purpose of the experiments 300 c.c. of water with 50 c.c. air space above were taken in all cases.

Experiment with Brahma-kund water.—The radio-activity of the water of *Brahma-kund* was determined by taking 300 c.c. of water with 50 c.c. of air space above ; air was bubbled through the water, after usual precautions, intro-

duced into the ionisation chamber, and the variation in the rate of activity observed :

TABLE LIV.—JANUARY 13, 1931.

Time of observation	Minutes	Calculated activity per hour
(1) 7.38 A.M. to 8.8.5 A.M.	30.5	41
(2) 8.38 " " 9.4.8 "	26.8	47.3
(3) 9.7 " " 9.33.2 "	26.2	48.5
(4) 9.38 " " 10.4 "	26	48.9
(5) 10.5 " " 10.31 "	26	48.9

The maximum was reached somewhere between 10.4 and 10.31, *i.e.*, between 2 hours 34 minutes and 3 hours; the gas emanation was introduced at 7.30 A.M., practically the same time as in the other cases.

Experiment with Makhdum-kund water.—The air bubbling through the 300 c.c. of water, as in the previous case, was introduced into the ionisation chamber at 2.10 P.M., and the following results obtained :

TABLE LV.—JANUARY 14, 1931.

Time of observation	Minutes	Calculated activity per hour
(1) 2.15 P.M. to 3.10 P.M.	55	20.9
(2) 3.11 " " 3.53 "	42	28.7
(3) 4.9 " " 4.50 "	41	29.5
(4) 4.51.5 " " 5.33 "	41.5	29
(5) 5.34 " " 6.17 "	43	27.9

The maximum was reached at or about 4.50 P.M., *i.e.*, after 2 hours and 40 minutes or shortly before 3 hours. This result affords conclusive proof of the presence of radio-active emanations in the *Makhdum-kund* water.

Experiment with Saptadhara water.—Employing the same method of observation, the experiment was carried out on January 16, 1931. The maximum activity per hour was thus found to be 7.4.

The radio-activities of the water from *Brahma-kund*, *Makhdum-kund*, and *Saptadhara* are thus in the ratio of 48.9 : 29.5 : 7.4 respectively. *The water from the spout of Makhdum-kund is thus four times as active as that from Saptadhara.*

9. ACTIVITY EXPRESSED IN EMANATION UNITS

In measuring the activity of different springs and pools two different electroscopes, with different constants, had been used, one in 1930 and another in 1931. As a result of this, though the same standard solution was used, yet the two electroscopes registered two different readings in 1930 and in 1931. The activity of *Brahma-kund* gas is, therefore, expressed by two different numbers: 136.02 in 1930, and 302.4 in 1931 (cf. pp. 333 and 338). This apparent difference disappears when we take into consideration the numbers expressing activity of the standard solution in the two respective years. A further correction is necessary, as in 1930 we worked with 60 c.c. of gas and 290 c.c. of water from *Brahma-kund*, while in 1931 the volume of gas taken was 50 c.c. with 300 c.c. of water. In calculating the activity of the gas alone it was also necessary to measure the activity of the water. This we could do after our observation in 1931.

Referring back to page 333, we find our standard solution contained 1.05×10^{-5} mgr. of radium = 1.05×10^{-8} grm. of radium. One Curie is the amount of emanation in equilibrium with 1 grm. of radium. Again 10^{-13} Curie per c.c. is 1 Eman.

We proceed now to express the activity of *Brahma-kund* water in Emans :

(1) *Brahma-kund* water taken, 300 c.c.

Activity observed . . . 48.9 divisions per hour.

Standard solution activity 488.6 divisions per hour.

Hence, in Eman units, the activity works out to :

$$\frac{48.9}{488.6} \times \frac{1.05 \times 10^{-8}}{10^{-13} \text{ Curie} \times 300 \text{ c.c.}} = 35.0 \text{ Eman.}$$

(2) *Brahma-kund* gas activity in 1931, after allowing for correction due to 300 c.c. of water :

$$\left(\frac{302.4}{488.6} \times \frac{1.05 \times 10^{-8}}{10^{-13}} - 35 \times 300 \right) \div 50 = 1089.7 \text{ Eman.}$$

Similarly

(3) *Brahma-kund* gas activity in 1930, after allowing for correction due to 290 c.c. of water :

$$\left(\frac{136.02}{191.17} \times \frac{1.05 \times 10^{-8}}{10^{-13}} - 35 \times 290 \right) \div 60 = 1075.9 \text{ Eman.}$$

It will be observed that, working with two different electroscopes and on days a year apart, the activity of the *Brahma-kund* gas was found to be practically the same.

Calculating in the same manner, the activities, in Eman units, for *Saptadhara* and *Makhdum-kund* spouts work out to :

(4) *Makhdum-kund* 21.1 Eman.

(5) *Saptadhara* 5.3 Eman.

It will be seen that the activity of *Makhdum-kund* spout water is about 4 times that of *Saptadhara*. Comparatively speaking, the activity of *Makhdum-kund* is of the same order as that of *Brahma-kund* water. It was not possible to collect gas from the *Makhdum-kund* pool or the *Makhdum-kund* spout on account of masonry covers and other obstacles. I have no doubt that if gas were collected from the *Makhdum-kund* source the activity would be found of the same order as that of the *Brahma-kund*.

Meyer and Schweidler give, in emanation units, a list of radio-active waters in different parts of the world with the names of the authors.¹ Amongst these, reference is made

¹ Meyer and Schweidler, *Radioaktivität*, pp. 568-569.

to Tuwa, Bombay, observed by A. Steichen. Reference may also be made to other works of A. Steichen, Sierp,¹ and others, as also to Mann and Paranjape,² on Hot Springs of Ratnagiri Districts. I shall quote here only a few typical instances from Meyer and Schweidler :

Tuwa, Bombay	. 330-620	(Steichen and others)
Aix-les-Bains		
	(France) 205	(P. Curie and A. Laborde)
Karlsbad (Muhl-		
brunnen)	115	(St. Meyer and H. Mache)
„ (Sprudel)	0.4	„ „
Baden-Baden		
	(Buttquelle) 290-450	(C. Engler and H. Sieveking)
Manitou (Color-		
ado, America)	50-305	(O. C. Lester).
Guergour (Algeria)	49-1220	(J. Pouget and D. Chouchak)
Arima (Japan)	. 138	(D. Isitani and K. Manabe)

There are others where the activity is very high, as, for example, in Grubenwasser (St. Joachimsthal), 7500 Eman. These high activity figures are indicative of radium mineral deposits near by. Deposits at Joachimsthal are the most important in the world, with which M. and Mme. Curie carried out their original investigations.

Summarising the results obtained with Rajgir samples, we get :

<i>Saptadharma</i>	. . .	5.3
<i>Makhdum-kund</i>	. . .	21.1
<i>Brahma-kund</i>	. . .	35.0
<i>Brahma-kund</i> gas	. . .	1075.9-1089.7

They compare favourably with the list quoted above. There can be no doubt that the Rajgir waters are radio-active and that the gas evolved is even more so. The commercial

¹ Steichen, Sierp, and others, *Trans. Bombay Med. and Physio. Soc.*, 1911, vol. xv, no. 1; *Ind. Med. Gaz.*, 1913; *Phil. Mag.*, 1916.

² Mann and Paranjape, 'Hot Springs of Ratnagiri Districts,' *Jour. Roy. Asiatic Soc.*, 1916.

exploitation of water transported to a distance for curative purposes would appear, however, to be of problematic value, since the radio-active emanation is liable to spontaneous decay.

I take this opportunity of expressing my grateful thanks to Sir J. C. Bose for suggesting the work and for encouragement and advice throughout the investigation.

I. A. R. I. 75.

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